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The National Institute for Research in Dairying, University of Reading

and

J. A. B. SMITH, Ph.D., D.Sc., F.R.I.C.
The Hannah Dairy Research Institute, Kirkhill, Ayr

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COMPILED FROM WORLD LITERATURE

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THE COSTANTINO GORINI FOUNDATION

A foundation has been created honouring the name of Costantino GORINI, well-known dairy bacteriologist who died in September, 1950 in his native city of Milan, Italy. It was formed under the auspices of the Lombardo Institute of Sciences and Letters of Milan for the purpose of assisting research workers engaged in dairy science and technology, especially in the field of microbiology.

Periodically an international competition will be held and a prize awarded the candidate who has demonstrated the best results in improving the production and uses of milk and dairy products in the interest of human nutrition.

Competition is now open and will close on **December 31, 1952**. Entries should be sent to INSTITUT LOMBARDO, VIA BRERA 28, MILAN. An entry shall consist of a letter of application, supporting reprints of published work and an account of the research work completed since 1949. The reprints and the letter of application may be written in one of the following languages: Italian, French, English, Spanish or German. The account of the work itself must, however, be written in Italian or French.

The prize is 100,000 liras.

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457. THE SALE OF SURPLUS CALVES IN GREAT BRITAIN, 1940–50

BY R. PHILLIPS
University College of Wales, Aberystwyth

(With 6 Figures)

It is of general interest to examine the sales of surplus calves to the Ministry of Food. Phillips and others (1, 2, 3, 4, 5) have shown that there are many features to this trade since the inauguration of the Fat Stock Scheme in 1940. The sales increased annually to 1946, when the introduction of the calf-rearing subsidy brought about a gradual reduction in the deliveries. The development of dairying in this country during this period with the priority for winter milk affected both the annual numbers sold as well as the seasonality of the sales. Consequently, the autumn deliveries increased rapidly at the expense of those of spring and summer. Phillips, Davies & Brown (2, 3) have also shown that the rates of autumn calvings throughout this country increase gradually from west to east, except that in the peninsulas of west and north Wales the reverse trends are obtained. The varying rates of autumn calving from west to east corresponds with a parallel increase from west to east in the average yield of milk per recorded cow, as shown by the reports of the National Milk Records of the M.M.B.

The records of the monthly calf births in England and Wales, as shown in the quarterly returns since 1945 and the cattle population figures correspond in many respects with the records of the sales of surplus calves. Phillips (5) has also shown that the quality of the veal calves in west Wales varies with the season and with other features of the traditional systems of farming, whilst the rate of winter conceptions in cows seems to be influenced by the climatic conditions.

The paper provides a record of the sales of surplus calves during the period 1940–50 and gives information on the changes in the seasonality of the sales as well as on the quality of the calves as determined by the official graders of the Ministry at the collecting centres.

(1) ANNUAL CALF SALES

During the eleven years, 1940–50, Great Britain produced an average of nearly one and a quarter million calves each year for sale to the Ministry of Food. The annual output, however, varies enormously from 861 thousand in 1940 to nearly 1449 thousand in the peak year 1946.

The annual figures are illustrated in Fig. 1, and for convenience of comparison the annual population (4 June) of cattle under 1 year old is also given.

The graph shows rapid increases during the first 4 years of the period, and after a slight reduction in 1944, the increases continue to the peak year 1946. After that date there is a continuous fall to 1949 but a sharp increase again in 1950.

The increases during the first 7 years correspond with the development of dairying, whilst the fall in 1947 is partly accounted for by the severe winter conditions of that year

and partly, as in subsequent years, by the economic encouragement of the calf-rearing subsidy. The populations (4 June) towards the end of the period are the reverse of the sale figures. It should be remembered that the total national dairy and breeding herd has increased nearly 10% during the period under review, so that the annual calf crop would also have increased accordingly.

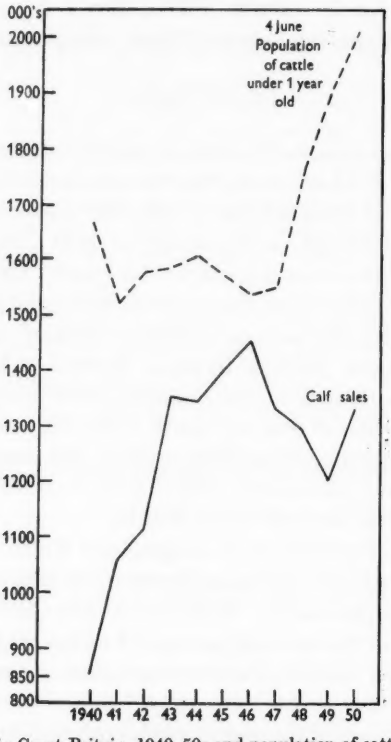

Fig. 1. Annual total calf sales in Great Britain, 1940–50; and population of cattle under 1 year old on 4 June.

(2) SEASONALITY OF CALF SALES

Phillips(4) has shown that the seasonality of calf sales and the seasonality of calf births are similar, and that for the period 1943–7 the months of peak sales and births are March and October. It has also been shown that these peaks balance one another like a see-saw. Thus, when the October peak is high the March one is low and vice versa.

For the period 1940–50 the monthly sales follow the same trends, although in the early years the position was not stabilized as it became later on. In order to exclude annual variations, the graph in Fig. 2A has been made on two 3-year averages, 1941–3 and 1948–50. The monthly percentages of calf sales show the differences between the two periods, i.e. the beginning and the end.

The curve for 1941–3 shows a spring peak in April, which is higher than the autumn peak in October. Actually, April was the peak month for the first 4 years of the period (1940–3). There is also a small July peak in the 1941–3 curve, showing that in the early years of the rapid expansion of dairying the calvings had not been stabilized for winter

milk production. In the 1948–50 curve, however, the autumn peak in October dominates the whole picture, it is far above that of the spring peak in March. This has been secured at the expense of both the spring and summer calvings. Indeed by this time summer calvings are of small dimensions. High autumn calvings imply December to March conceptions, and this is in general confirmed by the service returns of the A.I. centres.

The monthly numbers of calves sold throughout the period 1940–51 are illustrated in Fig. 3, Sheets 1 and 2. The monthly numbers of bobbies are also given.

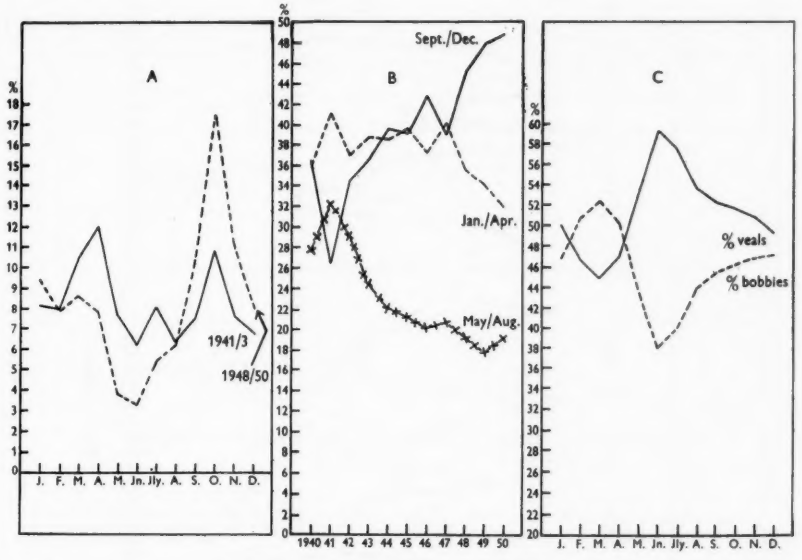

Fig. 2. A. Seasonality of calf sales in Great Britain: comparison of two 3-year periods, 1941–3 and 1948–50. B. Annual calf sales in Great Britain: three 4-monthly periods. C. Comparison of bobbies and veals in Great Britain: monthly distribution of sales, 1941–50.

It is seen that the annual numbers have increased, but whereas there was some irregularity in the pattern of the graphs in the early years, it had become more definite in shape from 1944 onwards.

In the early years of the decade, there were 3 months of peak deliveries, April, July and October. The July peak, however, gradually disappeared, and by 1945 it was very small in size. By 1945 also, the April peak had moved backwards into a March peak, but in the course of the years a January peak had been established in increasing dimensions. In 1945 it was equal in size to the March peak, and in the subsequent years, apart from 1949, it had become bigger than the March one. The main feature of the records, however, has been the ever increasing volume of the calf trade in October; this peak invariably stands alone each year. In 1950 the October deliveries became nearly five times that of June and three times that of March or January. This is a reflexion of the increasing importance of dairying in Great Britain, and of the production of winter milk within that system.

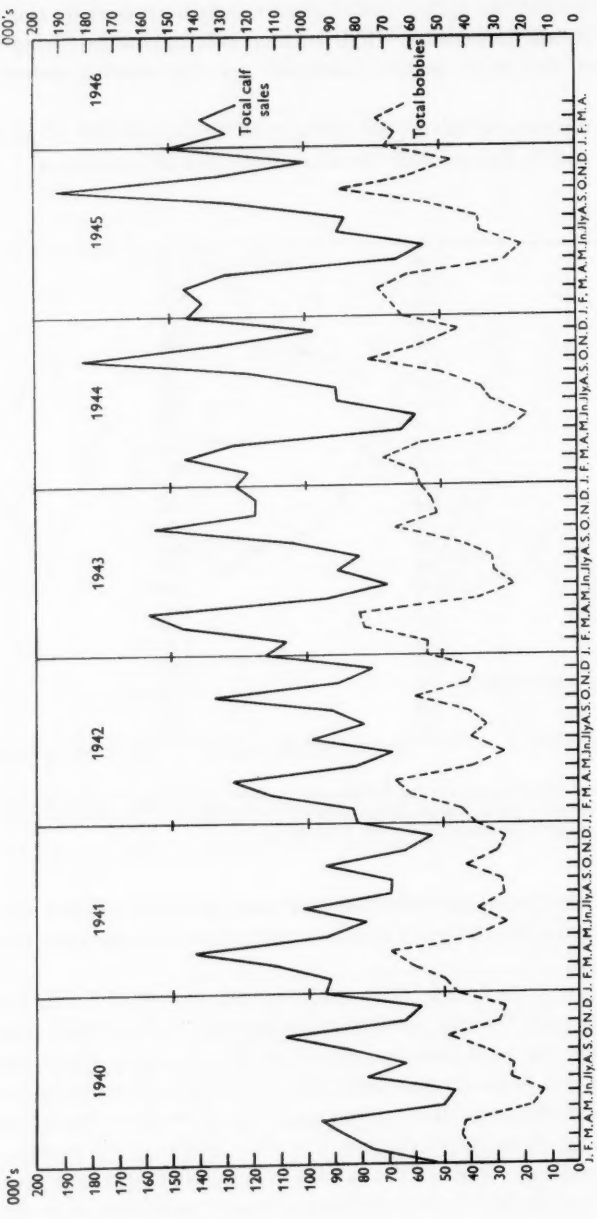
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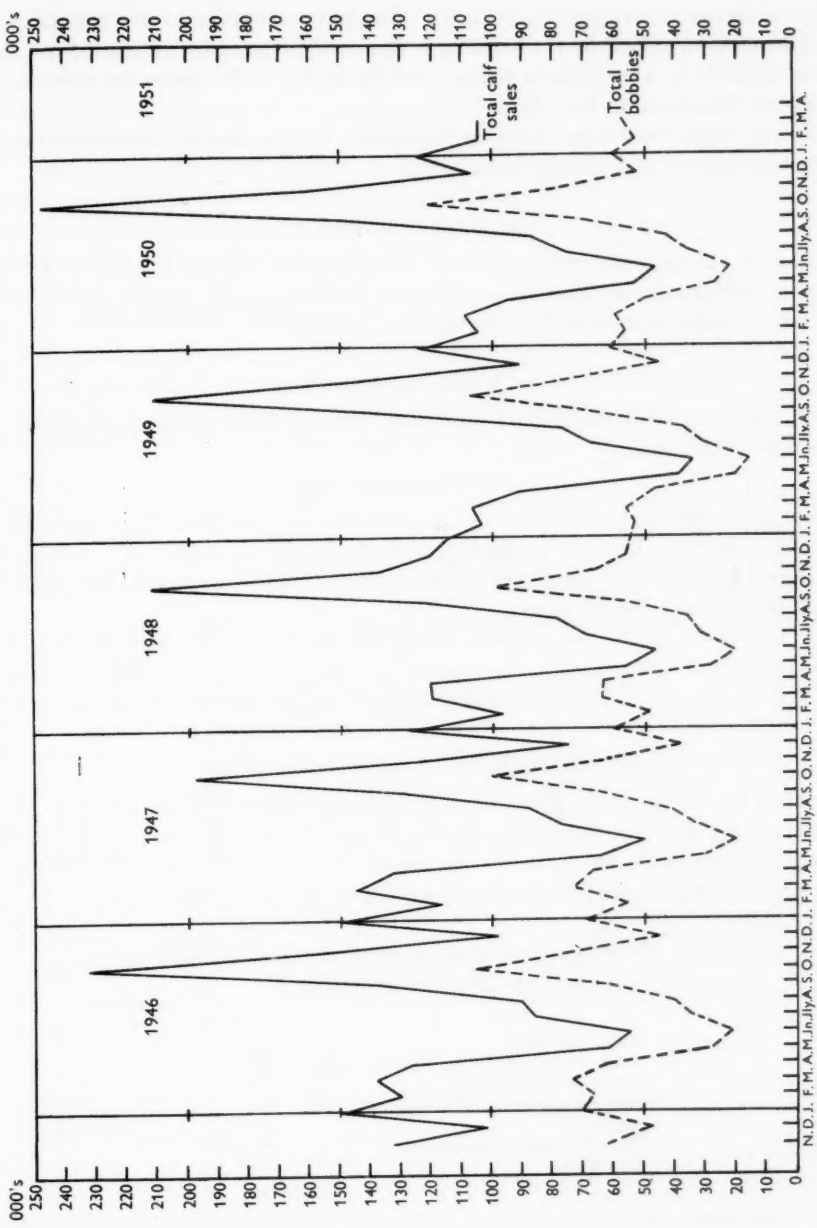
Fig. 3. Sheet 1.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Total bobbies
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Fig. 3. Sheet 2.

(3) ANNUAL TRENDS IN CALF SALES

Phillips & Davies(1) have previously shown that the simplification of the seasonality of calvings can be expressed by the percentage figures of the sales during three 4-monthly periods, January to April, May to August, and September to December for each year. These are illustrated in Fig. 2B.

After the initial disturbance of the wartime policy in 1940 the country strove towards a definite system of herd management.

(*a*) *January to April*

Very little change is shown from 1940 to 1947, but after that year there was a rapid decline in calf sales during this season of the year, showing that the calves kept for rearing were taken mainly from those born during these months.

(*b*) *May to August*

From 1941 the curve shows a downward trend indicating that summer calvings become fewer from year to year.

(*c*) *September to December*

From 1941 onwards this curve shows a continuous rapid rise from 26% in that year to nearly 50% in 1950. The only irregularity is shown by the decline in 1947. That severe year caused havoc to many aspects of animal production, and it delayed the calvings from the autumn to the following winter.

It is seen that by 1950, the calf sales during the three 4-monthly periods, are widely spread about; only 20% occur in May to August, 32% in January to April and 48% in September to December. The calvings are obviously set for high winter milk production.

(4) THE CONDITION OF THE SURPLUS CALVES

The calves purchased by the Ministry of Food through the collecting centres in Great Britain are classified by the official graders in accordance with their condition. The average percentages for each class for the nearly 13 million calves accepted during the 10 years 1941–50 (the 1940 figures are not available) are shown in Table 1.

Table 1. *Percentage distribution of calves purchased by the Ministry in Great Britain during* 1941–50

Class	Percentage	
Veals, 1st quality	6·0	50·8
Veals, 2nd quality	12·7	
Veals, 3rd quality	32·1	
Other strong calves or 'Stirkies'	0·3	49·2
Bobbies	46·7	
Unfit for human consumption	0·5	
Casualties	1·6	

The table shows that the calves delivered consist of approximately equal proportions of vealers and bobbies and others. By far the highest proportion of the veal calves are of the third quality. Bobby calves account for 46·7% of the total, whilst the remaining three classes are only of very minor dimensions. The other strong calves or 'stirkies'

(0·3%) are in reality big sized vealers and were included in that group during 1940. Calves unfit for human consumption are 0·5%, whilst casualties were three times as many (1·6%).

Since 3rd quality vealers are practically of the same condition as bobbies although somewhat bigger in size, then these two classes account for 78·8% of the total. In other words, no special effort was made to produce good veal calves in Great Britain during this period, for the reasons that many of the bobby calves are small dairy bred types (Jersey or Ayrshire) which could not easily be brought into the veal class by further feeding, and secondly because the price of milk has been at a higher level than that of veal, which by the way, remained unchanged until 1951. Both the breeding and economic policies have reacted adversely on the production of veal.

(5) ANNUAL CHANGES IN PROPORTIONS OF VEALS AND BOBBIES

Fig. 4D shows the proportions of veals in the total calf sales for each year. There was a gradual, although slight, improvement up to 1944, but after that year the proportion of vealers fall considerably. Fig. 4D also shows the annual proportions of 3rd quality vealers in the total veal calves. This proportion has increased regularly from 50% in 1941 to nearly 70% in 1950.

Both these graphs indicate a gradual decline in the effort to produce good quality veals (1st and 2nd quality). Phillips (5) has previously shown that in west Wales the intensification of dairying has effected a reduction in the supplies of good veal calves, which implies a deterioration during the past 10 years in the quality of calf-rearing. Calves have become surplus by-products of milk production, and the sooner they are disposed of the better the financial returns.

(6) SEASONALITY OF VEALS AND BOBBIES

Fig. 2C illustrates the monthly distribution in the proportions of veals and bobbies. The proportion of veal calves (the three qualities) is lowest in March (44%), then increases rapidly to a peak in June (58%), and it gradually falls from this month to the following March, remaining at nearly 50% in December and January.

The proportion of bobbies shows the reverse pattern, being highest in March and lowest in June.

These results are of course mainly the influence of the monthly price levels for milk. When milk is relatively cheap in May and June then veal is produced more abundantly. The influence of the seasonality of calvings on these proportions is slight, although the March peak in bobbies coincides with the March peak in sales; but there is no corresponding relationship in October.

(7) QUALITY IN VEALS

The average proportions of 1st, 2nd and 3rd quality veals during each month (1941–50) are shown in Fig. 4A.

The curves in Fig. 4A show that the proportion of 3rd quality veal calves follow the same pattern as the monthly distribution of bobbies (Fig. 2C), whilst that of 1st quality veals has the same pattern as that of veals in the same graph. The proportion of 3rd quality veals falls to a minimum in June, whilst that of 1st quality is highest in June/July. The proportion of the 2nd quality veals remains more level at nearly 24% of the total veals, although it shows a slight rise during the summer months. When milk is relatively

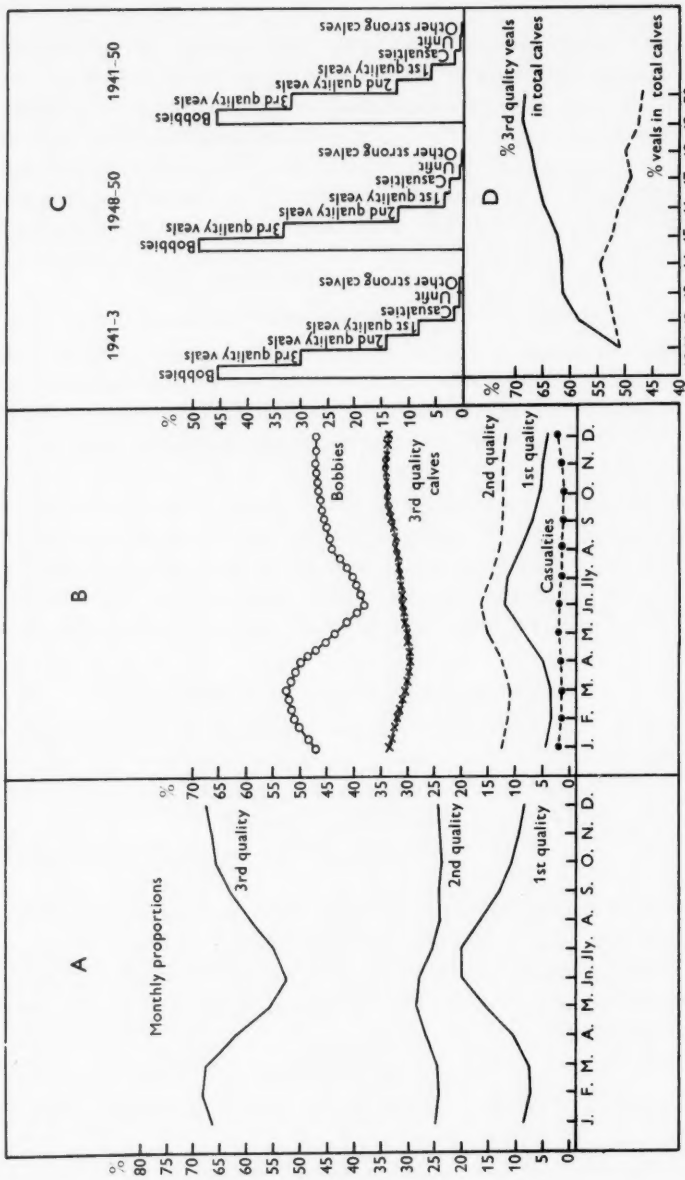

Fig. 4. A. Monthly percentage distribution of 1st, 2nd and 3rd quality veal calves in Great Britain, 1941–50. B. Percentage monthly distribution of calves in Great Britain according to class, 1941–50. C. Percentage distribution into classes of calf sales in Great Britain, 1941–3, 1948–50 and 1941–50. D. Annual percentages (Great Britain).

cheap, it results in the increased production of 1st quality veals at the expense of the 3rd quality. Thus the production of veal calves of 1st class quality depends on cheap milk in summer.

(8) PERCENTAGE MONTHLY DISTRIBUTION OF CALVES OF EACH CLASS (1941–50)

Fig. 4B illustrates the average distribution during each month according to class. The curve for bobbies follows the normal lines previously indicated (between 40 and 50%). The proportions of 3rd quality veals remains fairly constant throughout the year (the numbers of course fluctuate enormously according to the seasonality of calf sales, Fig. 2), although there is a slight trough in spring and then a gradual rise to the following December. The 1st and 2nd quality veals also follow the pattern, previously described, of a mid-Summer peak in the proportions at the time of minimum deliveries (Fig. 2A).

The proportion of casualty calves is low throughout (1–2%), but there is an indication of a higher incidence in December and January than during the remainder of the year.

(9) PERCENTAGE DISTRIBUTION OF THE DIFFERENT CLASSES OF CALVES SOLD TO THE MINISTRY OF FOOD

A comparison of the average distribution of the classes is shown in Fig. 4C on a percentage basis for two 3-year periods, 1941–3 and 1948–50, and it is also shown for the whole 10 years, 1941–50, as in Table 1.

A comparison of the histograms for the two periods shows clearly the deterioration in quality. The proportions of bobbies and of 3rd quality veals are higher in 1948–50 than during 1941–3, and there is a corresponding fall in 1st quality veals. During this period the quality of the calves deteriorated, and this is illustrated for each month in Fig. 5A, where the proportions of bobbies, 3rd and 1st quality veals are shown for the two periods respectively.

The proportion of bobbies is higher from April onwards in the 1948–50 period and the gap is at its widest in mid-summer. In the 3rd quality veals also, the proportions in 1948–50 are higher than in the former period 1941–3, especially during the autumn and winter seasons; they are closest together in summer.

In regard to the 1st quality veals, however, the reverse process is found, since the proportions during 1948–50 are considerably lower than in 1941–3 and especially during the mid-season June/July.

All the graphs show that the quality of the calves has deteriorated during this 10-year period. The number sold during the months June/July in 1948–50 was much less than during the same months in 1941–3 (331,510 to 503,455), yet even with fewer numbers the quality has declined.

The seasonality of sales has practically no influence on the proportions of 1st quality veal calves.

(10) THE RELATIONSHIP BETWEEN CALF SALES AND CATTLE POPULATIONS

The annual mean population of the various classes of cattle have been derived from the quarterly returns of the Ministry of Agriculture. A comparison of these population figures with the annual sales of cows and calves to the Ministry of Food is shown in Fig. 6.

It is seen from the graphs in Fig. 6A that the mean population of cows (cows and heifers in milk and cows in calf not in milk) increased from 3153 thousand in 1940 to 3438

thousand in 1950, whilst that for cattle under 1 year old increased from 1602 thousand to 1976 thousand in the same period.

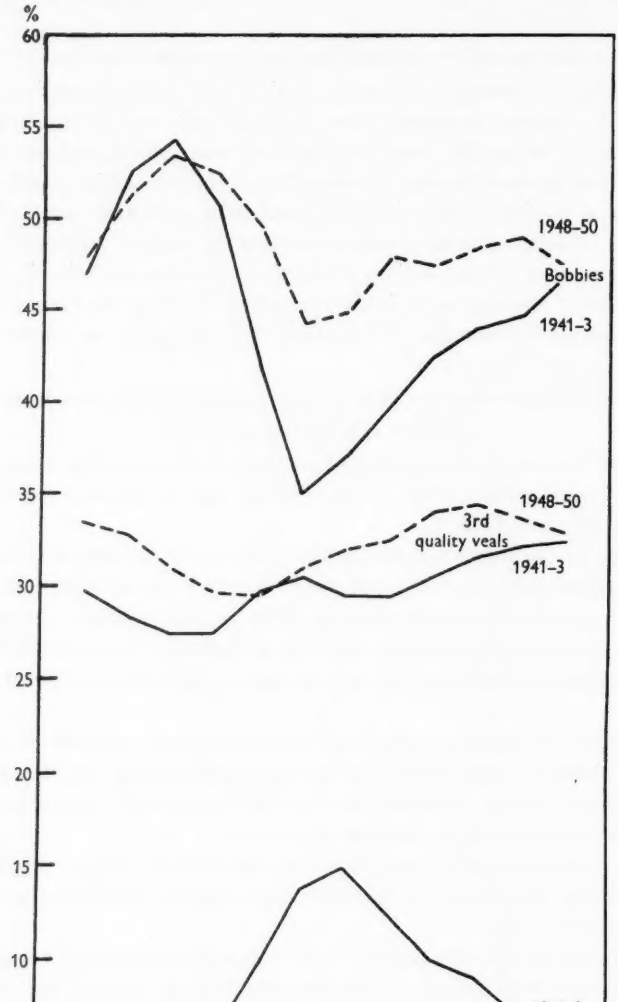

Fig. 5. Comparison of monthly differences between classes of calves sold in Great Britain during two 3-year periods, 1941–3 and 1948–50.

The estimated 'calf crop' has been obtained by adding together the annual calf sales and the mean quarterly population of cattle under 1 year old. The estimated calf crop is seen to have increased from 2463 thousand to 3305 thousand. For convenience, the sales

of cows to the Ministry of Food are also shown to have risen from 412 thousand to 588 thousand during the same period. The average sales of cows to the Ministry of Food represents 14% of the mean quarterly cow population.

In the intervening years there have been changes in trends. In calf sales, as has previously been shown, the increase continued rapidly to the peak year of 1946 and then declined to 1949, with a further increase in 1950. The mean population of cattle under

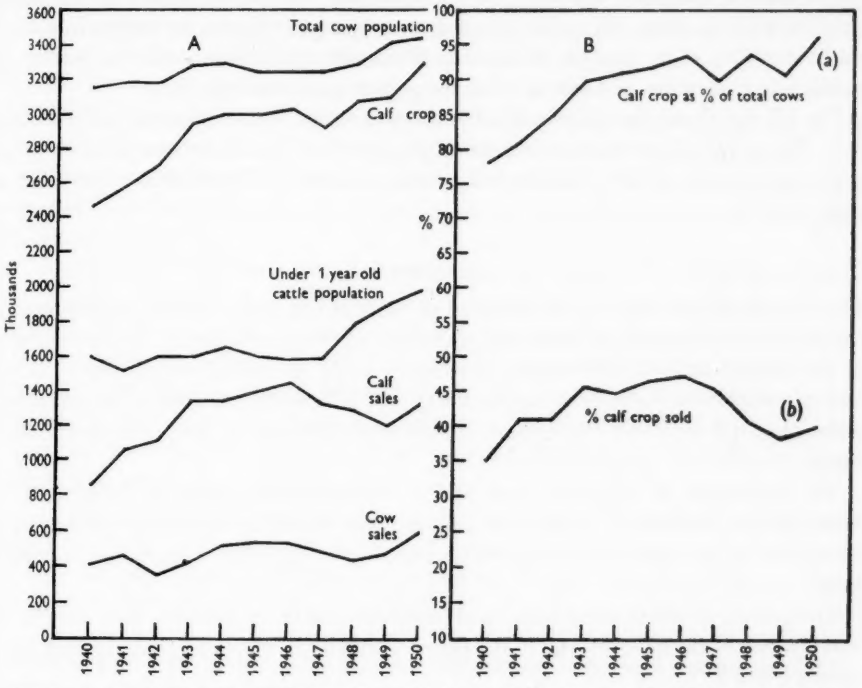

Fig. 6. A. Annual mean cow population and annual sales of calves and cows in Great Britain, 1940–50.
B. Percentages: (*a*) calf crop to total mean cow population; (*b*) calves sold to calf crop.

1 year old remained remarkably level until 1947, when due to the introduction of the calf subsidy scheme, the figure rose rapidly year by year. With the increase in the population of cows, the calf crop also increased continuously until 1947, when there was a sharp decline, owing no doubt to the severity of the weather conditions, which wrought such disastrous effects on cattle production.

It should be remembered that this estimated calf crop for Great Britain is probably too low, since the figures for England and Wales of the annual calf births are higher than the estimated calf crop for Great Britain (the calf births for Scotland have not been obtained) as shown in Table 2.

As the calf births are higher than the estimated calf crop, the calculated percentages

Table 2. *Estimated calf crop of Great Britain and the calf births of England and Wales*

	1945	1946	1947	1948	1949	1950
Estimated calf crop—Great Britain	2992	3033	2920	3083	3100	3305
Calf births—England and Wales	3009	3093	2936	—	—	—

shown in Fig. 6B are too low, and the position is therefore better than that ascertained by calculation. Fig. 6B gives the annual estimated calf crop as a percentage of the total cows. This figure at 78% in 1940 rose to 90% in 1943 and has remained above that figure for the remaining years. This may give an index of the fertility rates of the national herd and the average for the period 1943–50 is 92%, which compares favourably with that of the New Zealand Dairy Reports, especially since the annual calf births are probably higher than the estimated calf crop. On the other hand, the 1st calf heifers have not been included with the cows. Since the recorded mean quarterly figures for heifers in calf are only about 75% of the possible 1st calvings (Phillips(4)) their influence on the percentage would be considerable, and would bring the percentages down too low.

Fig. 6B also shows the actual calf sales as a percentage of the estimated calf crop each year. The graph shows that an increasing proportion of the calves were sold up to the peak year of 1946 at 47% but the calf subsidy scheme in all probability checked that tendency.

DISCUSSION

The average annual sales to the Ministry of Food of one and a quarter million surplus calves in Great Britain is an important by-product of the dairy industry. The development of the dairy industry in this country is reflected in the increasing annual sales of calves. The introduction of the calf-rearing scheme in 1947 reduced the numbers of surplus calves, although it did not affect the volume of milk production nor of the population of dairy cattle.

The economics of milk and meat and of winter/summer milk has influenced the seasonality of calf sales. Accordingly, during this period an increasing proportion of calves are sold in autumn and early winter each year, with October the month of greatest sales.

The general trends in dairy farming are also reflected in the sales for each month. The stabilization of the industry and of the policy within it are shown by the graphs of Fig. 3, Sheets 1 and 2.

On the other hand, the quality of these calf by-products, as determined by their grading at the collecting centres, is not high, since nearly one half of them are sold a few days old as bobbies, whilst two-thirds of the remainder are only a little better as 3rd quality veals. Fortunately, the proportions of calves unfit for human consumption, is low, whilst the casualties although numerically five times that of the unfit are also only 1·6% of the total. Unfortunately, the quality of the calves deteriorated from year to year as shown by the reduction in the proportion of calves classified as veals and an increase in the proportion of those of 3rd quality veals.

The seasonality of veal production follows the seasonal variations in the price of milk, since a higher proportion of veals and consequently a lower proportion of bobbies are produced in summer than in winter. The quality of the veal calves also improves during the summer, since there are then a higher proportion of 1st quality and a lower proportion of 3rd quality veals, but that of the 2nd quality remains stationary.

The peak month of veal deliveries is in October, although the highest monthly proportion is in June; this is in sharp contrast to the well-established traditional veal month of April of days gone by. The seasonality of calving does not seem to be related to the proportion of veals, although naturally it affects the volume of the trade.

The severe weather of 1947 affected both the numbers of the surplus calves as well as their quality as recorded in the returns of the collecting centres. This is in accordance with expectation as in that year the spring was late and milk scarce. Deaths in live stock of all classes was also higher than normal in that year.

SUMMARY

1. A record has been made of the volume of the trade in surplus calves in Great Britain for the period 1940–50; it has averaged one and a quarter million calves each year. The highest numbers sold in any year occurred in 1946 (Fig. 1).

2. The seasonality of calf sales showing an annual increasing volume of autumn calves (with October the peak month) is a reflexion of the development of dairying and of the priority for winter milk (Fig. 2A and Fig. 3, Sheets 1 and 2).

3. Of the total calves 46·7% were classified as bobbies and 50·8% as veals. The small remainder consisted of stirkies (other strong calves), casualties and those unfit for human consumption (Table 1).

4. Nearly 63% of the veals were 3rd quality and only about 12% of the 1st quality.

5. The seasonality of veal deliveries showed a maximum proportion in June (with a correspondingly lower proportion of bobbies in that month). The maximum numbers however correspond with the maximum gross deliveries in October (Fig. 2B).

6. There has been an annual deterioration in the quality of the surplus calves as indicated by: (*a*) the lower proportions of veals in the total during each year, (*b*) the higher proportions of 3rd quality veals in the total veals each year, and (*c*) the reduction in the monthly proportions of 1st quality veals.

7. An attempt has been made to relate the calf sales to calf births and populations of under 1-year-old cattle and of total cows.

8. It is estimated that the calf crop as a percentage of the total cows has improved since 1940, and for the last 8 years it has averaged 92%.

Grateful acknowledgement is made to the Ministries of Food and Agriculture for the material examined in this paper.

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458. CLINICAL MASTITIS IN SIX HERDS FREED FROM *STREPTOCOCCUS AGALACTIAE*

By F. K. NEAVE, MARY PHILLIPS and A. T. R. MATTICK
National Institute for Research in Dairying, University of Reading

The primary purpose of this paper is to draw attention to the high incidence of staphylococcus mastitis in certain high-class dairy herds under excellent management, the need for an examination of the possible influence of the type of milking machine on staphylococcus mastitis and the difficulty in preventing the occasional reappearance of *Streptococcus agalactiae* in 'free' herds.

Many herds free from *Strep. agalactiae* for a year or more have been reported by Plastridge (1), Cunningham *et al.* (2) and Stableforth *et al.* (3) to have little mastitis. But Cunningham *et al.* (2) present data which indicate that after the elimination of *Strep. agalactiae* there was little or no decrease in clinical mastitis in four of the nine herds examined, though there were fewer blind quarters.

While this paper does not claim that the increase in staphylococcus mastitis has any relation to the elimination of *Strep. agalactiae* from two of the herds, some investigators, Bean *et al.* (4), Johnson (5), Murnane (6) and Miller (7), indicate that an increase in staphylococcus infections followed a decrease of streptococcus mastitis.

Although some investigators have referred to the importance of staphylococcus mastitis, e.g. Mattick *et al.* (14), McEwen & Cooper (8) and Miller & Heishman (9), it would appear from the publications of Arthur (10), Stableforth *et al.* (3), Watts (11) and Berger & Francis (12), that staphylococci are responsible only for few udder infections and for few of the clinical cases reported by farmers in the areas investigated by these workers.

Plastridge *et al.* (13), Cunningham *et al.* (2) and Stableforth *et al.* (3) have given examples of the accidental reintroduction of *Strep. agalactiae* into 'free' herds by the purchase of infected animals and possibly from the contaminated hands of milkers who had handled cows in other herds.

OBJECT OF INVESTIGATION AND SOURCE OF DATA

It was desired to establish the time during which herds already free from *Strep. agalactiae* would remain free, what amount of clinical mastitis occurred and the types of organisms concerned in these cases.

The data were collected from six attested pedigree herds in Suffolk, which were under the same ownership and management. The four Guernsey herds, while physically separated, were in fact one herd from the point of view of breeding, and the same applied to the two Shorthorn herds.

Mattick *et al.* (14) described the early progress in the elimination of *Strep. agalactiae* from the two original herds D and E during 1939 and 1940, while Neave *et al.* (15) described an outbreak of clinical mastitis during 1943 in herd E, which was associated with *Strep. uberis* and a high vacuum in the milking machine.

The other four herds were founded between June 1941 and January 1948, mainly with heifers from farms D and E and some in-calf heifers from other herds.

MANAGEMENT OF THE HERDS

A general description of the management of the herds has been given by Brookes (16).

Calves were raised on raw milk from the herd in which they were born; they were then grouped according to age, and usually reared elsewhere. About a month before calving they were drafted to the various herds. A proportion of heifer calves came from other estates of the same owner, where the herds were known to be infected with *Strep. agalactiae*.

Milking was by movable or fixed bail milking machine and the cows were stripped by hand until about the end of 1949 when five of the farms adopted machine stripping.

Hypochlorite was used, according to the method of Harrison & Mattick (17) as a disinfectant for washing udders and dipping teat-cups before milking each cow. This method has been used on farms D and E since 1939 and on the other farms since the herds were formed. Some modifications, described in the following pages, in this routine were made on certain farms.

There was little change in the permanent milking staff during the survey but when a change occurred a record was kept. There were, however, some changes of temporary staff, especially during the war years, and there was a relief milker who might have carried infection from farm to farm.

METHODS

Clinical mastitis. A strip-cup was used twice a day before milking each cow. The cowman made a note of cases of clinical mastitis and injuries to the udder and teats, and a record of these occurrences was kept. Such cases were reported to the veterinary surgeon who confirmed the condition and carried out treatment. The injuries were chiefly from horning, which caused the appearance of blood or blood clots in the milk. They were treated by means of udder infusions and/or sulphanilamide, administered orally, but such cases have been excluded from the data given in this paper. Some injuries were caused by barbed wire.

Before treatment started, samples were taken and dispatched to Reading. Until 1944, 1 oz. single-quarter samples were sent by rail or by post in the sterile bottles provided. From 1944 the milk samples were streaked, by a trained person, on to aesculin blood agar in bottles (18) before dispatch by post, and from January 1947 milk was taken direct from the teat on to blood agar in bottles (S.P. units) (19).

Herd examinations. In 1941 and 1942 separate quarter samples of milk were taken from all animals in herds D and E at intervals of about 3 months. They were subsequently taken less frequently, and in some years not at all unless *Strep. agalactiae* was recovered from samples of S.P. units received by post. The first two columns of Tables 1–3 show the number of herd examinations and whether samples were examined by direct plating or by enrichment methods.

The method of examining the milk samples before 1942 has been described previously (14). In 1942 enrichment methods (20) only were used. From 1943 enrichment methods and/or direct plating methods were substituted. For a period of 3 years the plating of 1 ml. of a one-tenth dilution of milk in aesculin blood agar was compared with surface-streaked plates, using wire loops of various diameters. Surface plating was eventually adopted using a flat wire loop (on a bent handle) delivering about 0·025 ml. of milk. Two loopsful

from each sample were spread on a Petri dish of aesculin ox blood agar, i.e. one loopful to each half of the dish, or a total of about 0·05 ml. of milk.

Teat swabs, swabs of sores on the teats, of milkers' throats and hands (including hand washings) and swabs of door and churn handles were cultured as previously described (20).

A record was kept of all teat sores and abrasions and of eroded teat orifices.

Organisms were identified by the usual methods (14, 15), except that staphylococci were infrequently tested for coagulase production until 1945. The results of this test however, only confirmed as staphylococci those organisms which had been regarded as such on the basis of type of growth, colour and haemolysis. Few coagulase positive staphylococci were found which did not produce $\alpha\beta$-zones on ox-blood plates.

Intramammary infections. Even with careful disinfection of the teat orifices by rubbing with cotton-wool soaked in a hypochlorite solution it is of course not possible to sterilize the skin. For this reason, because sampling could not be frequent and for comparative purposes, it was necessary to decide on the minimum colony count to be accepted as indicating an 'infection'.

A colony count of ten or more (i.e. about 200 per ml.) staphylococci or streptococci per blood plate was the criterion finally adopted. A few exceptions were made when there was a pure culture of less than ten colonies. Of the plates showing streptococci or staphylococci, 18 and 24% respectively had less than ten colonies per plate; generally one or two colonies only. All these, with the few exceptions mentioned, were disregarded when assessing the number of 'infected' animals. The criterion, in our experience, does not appear to be too lenient or too severe, as persistent infections very seldom yield less than 200 colonies per ml. (1, 21, 22, 30), and with care contaminants, e.g. staphylococci from external skin infections, can be kept below 200 colonies per ml. of milk.

Enrichment methods and teat swabs were, of course, used solely to detect the presence of *Strep. agalactiae* in the herds. As direct plating of 0·05 ml. of milk frequently reveals contamination from the exterior of the teat, it is not possible to accept the results of enrichment methods as indicative of intramammary infection, especially when 5–30 ml. of milk are incubated. Successive herd examinations of *Strep. agalactiae* free herds showed that the number of milk samples yielding other streptococci after, but not before, incubation for 24 hr. at 37° C., fluctuated between 10 and 60%. These results bore no relationship to the number of intramammary infections as normally diagnosed (1, 21, 22, 30). Most of the teat abrasions were heavily infected with *Strep. dysgalactiae* and haemolytic staphylococci.

We have found no acceptable evidence of the occurrence of so called 'light infections' to be detected only by enrichment methods or that infections are revealed earlier by using such methods. Temporary infections unquestionably exist, but are of little significance unless accompanied by clinical symptoms.

Strep. agalactiae. From 1939 to 1944, if *Strep. agalactiae* was recovered from the milk of cows, the animals were removed from the farm (14). There were some exceptions, mentioned in the text, to this rule. Between 1944 and 1948 there were no *Strep. agalactiae* infections. From 1948 to 1950 those cows which were considered to be infected, and many of those yielding *Strep. agalactiae* by enrichment methods only, were treated with penicillin.

RESULTS

The occurrence of Lancefield group B streptococci. The frequency of the recovery of group B streptococci from the udder and from milkers' hands and throats is shown in Tables 1–3.

Herd D. This herd (Table 1) was probably free from true intramammary infections from May 1940 to 1950, because the four cows from which *Strep. agalactiae* was recovered before February 1941 (14) were shown by subsequent tests to be uninfected. The streptococci, recovered from only one sample in each case, probably came from the exterior of the teats.

In March 1942 the milk of a heifer was found by enrichment methods a few days after calving, to contain *Strep. agalactiae:* she had been stripped out by hand in the normal way. Subsequent milk samples were negative after she had been removed from the herd in March. It is possible that her teats and those of the cow with an infected sore found to be positive in June were contaminated with group B streptococci from the hands of milker S. This is thought to be the most probable explanation in view of our experience on other farms.

The infected sore teat was treated with tincture of iodine twice a day until it healed. Subsequent samples and swabs were negative, although the milker's hands were still positive in August. The three strains from the milk, hands and sore were of the same serological type.

Herd E. This herd (Table 1) was thought to have been free from udder infection since November 1940, because the five cows found to be positive early in 1941 were subsequently found to be negative. Only very few group B streptococci had been recovered from one sample from each cow.

Strep. agalactiae was recovered from the teats of cow 1446 on two occasions early in 1942. It had previously, in 1940, been isolated from her milk by enrichment methods, but many other tests were negative. In view of these findings the cow was moved to Reading and a supernumerary gland with a small orifice was discovered. Fluid from this gland was examined on several occasions and found to be heavily infected with *Strep agalactiae.*

Before the removal of the supernumary gland, one of seventy-eight milk samples from the normal glands was positive (five colonies) by direct plating of 0·2 ml. of milk and nine of 127 enriched samples. Many teat swabs were also positive, and two of three teat abrasions became heavily infected. There was no detectable difference in the composition of the milk of the four quarters, and it was concluded that the normal quarters had remained free from established intra-mammary infections. After the supernumary gland had been successfully sealed off, subsequent milk samples and teat swabs were negative. This cow had her first calf in November 1939 and the infection undoubtedly occurred before measures to free the herd from *Strep. agalactiae* had been completed.

It is probable that the good hygiene practised on the farm, together with the apparent resistance to infection of the normal quarters of the cow, prevented any new infections in the 12 months prior to her removal from farm E.

In November 1944, a temporary milker was found to be a throat carrier and, although she assisted in the milking for 3 months, group B streptococci were recovered from the teats of only one cow during this period. The milker was fond of milk and lived in a hostel supplied with milk from a farm known to have mastitis.

Table 1. *The recovery of Lancefield group B streptococci from cows and milkers*

Date	Milk samples: Direct plating	Milk samples: Enriched (neg. direct)	Teat swabs	Clinical cases	Swabs from milkers: Throat	Swabs from milkers: Hands	Utensil swabs	Remarks
Farm D. Guernsey herd founded in 1937								
1941 (50–70 cows tested at each visit)								
Feb.	0	0	—	0	—	—	—	—
May	0	0	—	0	—	—	—	—
Aug.	0	0	0	0	—	—	—	—
Nov.	0	0	0	0	—	0/4	—	—
1942								
Feb.	—	0	0	0	0/4	0/5	—	Includes milker S.
Mar.	—	1 H.	—	0	—	0/3	—	Heifer just calved
Apr.	—	0	0	0	—	1/3	—	H. strain, milker S.
May	—	0	0	0	—	0/2	—	—
June	—	0	1 H.	0	—	1/4	—	Both H. strains, infected sore on teat
Aug.	—	0	0	0	—	1/3	0/7	H. strain, milker S., June and Aug.
Dec.	—	0	0	0	0/1	0/6	—	Includes milker S.
1943								
Mar.	—	0	0	0	0/2	0/5	—	Includes milker S.
May	—	0	0	0	0/1	0/3	—	Includes milker S.
Sept.	0	—	0	0	—	—	—	—
1944								
Mar.	0	—	0	0	—	—	—	—
Nov.	0	—	0	0	—	0/4	—	Includes milker S.
1945								
June	0	—	0	0	0/3	0/5	—	Includes milker S.
1946								Milker S. left farm
Jan.	0	—	—	0	—	—	—	—
June	0	—	0	0	0/3	0/4	—	—
Oct.	0	—	0	0	—	—	—	—
1947	—	—	—	0	—	—	—	—
1948	—	—	—	0	—	—	—	—
1949	—	—	—	0	—	—	—	—
1950								
Nov.	3γ	—	6γ	0	—	—	—	3 cows: 2 LF quarters, 3 LH quarters

Cows positive; direct plating: 3 γ-strains, 1950
Cows positive; enrichment *only*: 1 H. strain, 1942
Cows positive and clinical: 0
Cows positive and treated: 3 γ-strains (Nov. 1950)

Farm E. Shorthorn herd founded before 1939

1941 (40–60 cows tested at each visit)								
Jan.	0	2	—	0	—	—	—	Both cows left in herd and subsequent tests negative
Apr.	3	0	—	0	—	—	—	These three 'sold'* in May, 3–10 previous tests negative and subsequent tests (2–3) negative
June	0	—	—	0	—	—	—	—
Aug.	0	—	—	0	—	—	—	—
Dec.	0	—	—	0	—	—	—	—
1942								
Feb.	—	0	1γ	0	—	0/2	—	Cow 1446 was positive by enrichment in Oct. 1940, but subsequently negative until Feb. and April 1942. It was then removed (see text)
Apr.	—	0	1γ	0	—	0/2	—	—
May	—	0	0	0	—	—	—	—
June	—	0	0	0	—	0/2	—	—
Aug.	—	0	0	0	—	0/2	—	—
Dec.	—	0	0	0	—	0/2	0/2	—
1943								
Mar.	—	0	0	0	0/2	0/3	—	—
May	0	0	0	0	—	0/3	—	—
Sept.	0	—	0	0	—	0/3	—	—
1944								
Mar.	0	—	0	0	—	0/4	—	New milker C. H. strain hands and throat
Nov.	0	—	1 H.	0	1/4	1/6	—	—
Dec.	—	—	—	—	1/4	0/5	—	Milker C., H. strain
1945								
Feb.	0	—	0	0	0/3	0/5	—	Milker C. left in Jan.
June	0	—	0	0	0/4	0/6	—	—
1946								
June	0	—	0	0	0/4	0/4	—	—
1947	—	—	—	0	—	—	—	—
1948	—	—	—	0	—	—	—	—
1949	—	—	—	0	—	—	—	—
1950								
Nov.	0	—	0	0	—	—	0/3	—
1951								
Mar.	0	0	—	0	—	—	—	—

Cows positive; direct plating: 3 γ-strains, 1941
Cows positive; enrichment *only*: 2γ-strains, 1941
Cows positive and clinical: 0
Cows positive and treated: 0

H. = narrow zone haemolytic strain. γ = non-haemolytic strain. 1/4 = one positive swab of 4 taken. 0 = *Strep. agalactiae* not found. LF = Left fore quarter of the udder.

* 'Sold' means removed to farm F where further tests were made.

Table 2. *The recovery of Lancefield group B streptococci from cows and milkers*

Date	Milk samples: Direct	Milk samples: Enriched (neg. direct)	Teat swabs	Clinical cases	Swabs from milkers: Throat	Swabs from milkers: Hands	Utensil swabs	Remarks
Farm no. 4. Shorthorn herd founded November 1941								
1942 (45–60 cows tested at each visit)								
Mar.	—	0	—	0	—	—	—	—
1943								
Mar.	—	—	—	—	—	0/1	—	Milker A.
May	—	—	—	—	—	0/3	—	Including milker A.
Sept.	—	0	—	0	—	—	—	—
1944								
July	—	0	—	0	—	—	—	—
1945								
June	—	0	—	0	—	—	—	—
1946	—	—	—	0	—	—	—	—
1947	—	—	—	0	—	—	—	—
1948								
Feb.	—	—	—	1	—	—	—	1st lact., H. strain
Mar.	—	—	—	1	—	—	—	H. strain
9 Apr.	0	—	—	0	—	—	—	—
29 Apr.	0	—	3 H.	0	0/2	2/3	2/4	Throat of A. not done, all H. strains
June	—	—	—	—	2/3	0/1	0/6	Milkers A. and D. both H. strains
Nov.	—	—	—	1	—	—	—	H. strain
8 Dec.	9 (3H.)	3 (1H.)	—	0	2/3	1/3	0/7	Throat of A. and D., H. strains
16 Dec.	4 (2H.)	10 (1H.)	—	0	—	—	—	8 new cases
1949								
Jan.	1γ	7 (2H.)	—	1γ	3/3	2/3	0/5	Throat and hands were H. strains
Mar.	0	1 H.	—	—	—	—	—	New case
June	—	—	—	2	—	—	—	Both new cases, both H. strains
July	—	—	—	—	1/4	0/4	0/5	H. strain, milker A.
Nov.	—	—	—	0	1/4	2/4	—	Throat of milker A., all H. strains
1950								
May	—	—	—	—	1/3	0/3	—	H. strain, milker A.
Nov.	1γ (new)	—	0	0	1/1	—	—	H. strain, milker A.

Cows positive; direct plating: 16 (7γ and 9H.)
Cows positive; enrichment *only*: 17* (12γ and 5H.)
Cows positive and clinical: 6 (1γ and 5H.)
Cows positive and treated: 15 (4 recurrences)

Date	Milk samples: Direct	Milk samples: Enriched (neg. direct)	Teat swabs	Clinical cases	Swabs from milkers: Throat	Swabs from milkers: Hands	Utensil swabs	Remarks
Farm no. 3. Guernsey herd founded June 1941								
1942 (25–65 cows tested at each visit)								
Feb.	—	0	0	0	—	1/3	—	Milker J., H. strain
May	—	0	0	0	—	0/2	—	Milker J.
June	—	0	0	0	—	2/3	—	Milkers J. and P., H. strain
July	—	—	—	—	—	0/3	—	—
Aug.	—	0	0	0	—	1/3	0/6	Milker J., H. strain
Dec.	—	0	1γ	—	—	1/3	—	Milker M., new person, γ-strain
Dec.	—	—	—	—	—	0/2	—	—
1943								
Mar.	—	0	0	0	0/3	0/5	—	Milkers M. and J. had left
Sept.	0	0	0	0	—	0/2	—	—
1944	—	—	—	0	—	—	—	—
1945	—	—	—	0	—	—	—	—
1946								
June	0	—	0	0	0/3	0/3	—	—
1947	—	—	—	0	—	—	—	—
1948	—	—	—	0	—	—	—	—
1949	—	—	—	—	—	—	—	Milker D. transferred from farm no. 4
June	—	—	—	1	—	—	—	3rd lact. γ-strain
July	—	—	—	2	—	—	—	γ-strains
Oct.	—	—	—	1	—	—	—	γ-strain
Nov.	—	—	—	1	—	—	—	γ-strain
1950								
Mar.	1	3	—	0	—	—	—	All γ-strains, new cases
July	—	—	—	1	—	—	—	γ-strain, new case
Nov.	2	—	3	0	—	—	—	All γ-strains, new cases

Cows positive; direct plating: 7 non-haemolytic strains
Cows positive; enrichment *only*: 2 non-haemolytic strains
Cows positive and clinical: 5 non-haemolytic strains
Cows positive and treated: 9 non-haemolytic strains (3 recurrences)

H.=haemolytic strain (double zone of haemolysis or partial haemolysis). γ=non-haemolytic strain. 2/3 etc.=2 positive swabs of 3 taken. New=not previously positive. 9 (3H.)=3 cows with haemolytic and 6 with γ-strains. 0=*Strep. agalactiae* not found.

* Seven of these were positive a second time but were never treated. They were subsequently all negative.

When last tested in July 1951 this herd was free from *Strep. agalactiae.*

Farm no. 4. This farm (Table 2) had been used to accommodate infected animals from farms D and E, but an entirely new herd was formed in November 1941 and was moved

Table 3. *The recovery of Lancefield group B streptococci from cows and milkers*

Date	Milk samples: Direct	Milk samples: Enriched (neg. direct)	Teat swabs	Clinical cases	Swabs from milkers: Throat	Swabs from milkers: Hands	Utensil swabs	Remarks
Farm no. 5. Guernsey herd founded August 1943								
1944 (35–68 cows tested at each visit)								
Mar.	0	—	0	0	—	—	—	—
Nov.	0	—	0	0	—	0/3	0/5	—
1945								
June	0	—	0	0	0/4	0/4	—	One throat swab, group A, streptococcus
1946								
June	0	—	0	0	0/3	0/4	—	—
1947	—	—	—	0	—	—	—	—
1948	—	—	—	0	—	—	—	—
1949								
Nov.	—	—	—	1γ	—	—	—	5th lactation cow, sold in December
1950								
Feb.	—	—	—	1γ	—	—	—	—
May	6	1	—	2	0/2	0/2	0/6	All γ-strains.
Nov.	7	—	8	0	—	—	—	All γ-strains, all new cases.
Cows positive; direct plating: 15 γ-strains Cows positive; enrichment *only*: 1 γ-strains Cows positive and clinical: 4 γ-strains Cows positive and treated: 15 (2 recurrences)								
Farm F. New Guernsey herd founded January 1948								
1948 (30–60 cows tested at each visit)								
7 Feb.	—	—	—	1?	—	—	—	10th lact. cow no. 540 LF from old herd, no sample sent.
12 Feb.	—	—	—	1?	—	—	—	6th lact., no sample sent
14 Feb.	—	—	—	1?	—	—	—	1st lact., no sample sent, positive on 16 Feb. however
16 Feb.	3γ	—	—	0	—	—	—	—
18 Feb.	—	—	—	1γ	—	—	—	—
Apr.	2γ	—	—	1γ	—	—	—	—
July	1γ	—	—	1γ	—	—	—	—
1949								
Nov.	—	—	—	1γ	—	—	—	No. 540 LF
1950								
Nov.	0	—	0	0	—	—	—	—
Cows positive; direct plating: 7 γ-strains Cows positive; enrichment *only*: — Cows positive and clinical: 4 to 6 Cows positive and treated: 8 (3 recurrences)								

γ = non-haemolytic strain. 0/4 = none of 4 swabs was positive. LF = left fore quarter of the udder. 0 = *Strep. agalactiae* not found.

into the farm on the same date as the eleven cows* in the old herd were removed. Nevertheless, group B streptococci were not recovered from this herd until a clinical case of a cow with sore teats occurred in 1948. A strain of *Strep. agalactiae* producing a double zone of haemolysis was recovered.

* Only two of these cows were found infected with *Strep. agalactiae* in September, although eight of them had harboured this organism before removal from herds D and E.

In the next 16 months at least nine cows were infected with the same or a similar strain, and five of them developed clinical symptoms. The cultures were found by Dr A. W. Stableforth either to be the same type or a closely related serological type as cultures recovered from the throats of two of the milkers on one occasion and from milker A. on many occasions. Most of these strains did not ferment lactose. Neither milker A. nor any other workers who were found to be carriers of this organism presented clinical symptoms of sore throat.

Group B streptococci were still present in the throat of milker A in July 1951, but no haemolytic strains were isolated from cows after June 1949.

It seems probable that the head cowman's throat (milker A.) was the source of the organism found in the herd, but it is not possible to determine when or how he became infected as the milkers' domestic supplies of milk came from the herd. The strain was serologically similar to another 'human' strain in the Weybridge collection.

A non-haemolytic strain of a different serological type was found in this herd in December 1948. Its source was not definitely known, but it probably came from herd F on the hands of the relief milker.

The figures at the foot of Table 2, though small in number, may indicate that the haemolytic strain of *Strep. agalactiae* was more pathogenic than the non-haemolytic strain, but less persistent on the skin of the teats.

Stripping by machine started in December 1949. In November 1950 there was still one infected cow in this herd, but none of the cows which had previously been positive only by enrichment methods, and which had not been treated, showed signs of infection.

Herd no. 3. This herd (Table 2) was apparently free from group B udder infections from 1941 until 1949, although streptococci of this group were recovered from the hands of two of the milkers (throat swabs were not examined) and from the teats of one cow, in 1942.

Herd no. 5. This herd (Table 3), founded in 1943, was also apparently free from *Strep. agalactiae* until 1949, when the streptococcus was recovered from a clinical case of mastitis.

In this herd separate sterile cloths were used for washing each udder, but stripping was done by hand throughout.

Group A streptococci were isolated from the throat of one milker.

Farm F. This farm (Table 3) had been used to house most of the infected cows (infected with both haemolytic and non-haemolytic strains) removed from the other herds. In 1948 a virtually new herd was formed, only a few of the old cows being retained.

Following the installation of a milking machine there was an outbreak of clinical mastitis (Table 4). Unfortunately, however, samples were not sent from three early cases (Table 3) which may therefore have been caused by *Strep. agalactiae* which probably came from the old cows from the original infected herd. No haemolytic strains were found. This herd was 'free' when tested in November 1950.

Although it is not certain, it is believed that the non-haemolytic strain of *Strep. agalactiae* was carried from herd F, to herd no. 4, by the relief milker, and from thence to the other herds. The relief milker had no contact with herds 4 and F before the new herds were established.

The order of recovery of the non-haemolytic strain of *Strep. agalactiae* which became established in the various herds was as follows: herd F in February 1948; herd no. 4 in December 1948; herd no. 3 in June 1949; herd no. 5 in November 1949; herd D in

November 1950; herd E still free in July 1951. Two to five cultures from each herd were typed at Weybridge. They were reported to be type 1*b* or a type closely related to type 1*b*.

Clinical mastitis. The number of clinical cases of mastitis reported each month from the six farms is shown in Table 4.

This table also shows the number of cows and quarters affected, the number of cows in milk each year, the average number of cows in milk each week and the average number of calvings. This latter figure gives an indication of the average age of the animals.

The average number of cows in milk each week of the year was estimated from the sum of the numbers in milk each eighth week, divided by 6. The average number of calvings was estimated from the sum of the number of calvings of each animal at each eighth week, divided by the sum of the number of cows in milk in these weeks.

For comparative purposes when the total number of animals in milk per year varies considerably, the number of clinical cases per year is expressed as a percentage of the average number of cows in milk per week.

Some of the changes which occurred in each herd through the years are recorded at the foot of Table 4, but other changes, e.g. those concerning milking staff and hygiene have been taken into consideration, as far as possible, when examining the results. One change, possibly significant, was in herd no. 4, which began changing over to a Guernsey herd in 1950. No Guernsey animals entered the herd, however, until August. In herd E penicillin was infused into all quarters of seventeen dry cows between January and September 1950.

The main purpose of Table 4 is to show the high incidence of clinical mastitis reported from five of the herds in certain years and the very low incidence in other years, and to demonstrate the influence of certain factors on these results.

From our own observations of the number of light quarters and from the observations of both the farm manager and the veterinary surgeon especially on farms 3 and 5, it must be concluded that some cases were not recorded. It is probable, too, that the other farms have been reporting more accurately since 1947, although their staff have always been very co-operative.

A number of changes or observations concerning the amount of clinical mastitis reported (Table 4) seem to be significant.

(1) The increase, following the change of milking machine on farm E in February 1948, and the further increase on reverting to the original type of machine in January 1950. This latter change was followed by a decrease in cases in March and April 1950 and a sharp rise in June and July, for which no explanation can be offered. There was, however, a marked fall in the number of clinical cases in 1951 (Table 7).

(2) The relative increase in infections in herd no. 4 after the milking machine had been changed in March 1947 and the decrease following the return to the original type of machine in January 1950.

(3) The outbreak of clinical mastitis in herd F following the installation of a milking machine in January 1948.

(4) There is no relationship between the amount of clinical mastitis reported from each herd and the year, except in herds no. 5 and D in the years 1946 and 1947. These two herds were originally managed as one herd, but were said to have been kept separate without interchange of staff since May 1945. There would therefore seem to be no simple explanation of the observations.

(5) There is no correlation between the average age of the animals in any one herd and the amount of mastitis reported. If, however, the results from all six herds are combined and graphed, a correlation is apparent between age and clinical mastitis.

(6) It is possible that the difference in the number of cases of mastitis reported in herds no. 4 and E is related to the difference between the average age of the cows in these herds. No such relationship, however, was found between herds no. 5 and D in 1946 and 1947.

(7) After changing their milking machines in 1947 and 1948, farms no. 4 and E were being milked with the same type of machine as the other herds. The difference in the incidence of mastitis in 1949 between these two Shorthorn herds and the Guernsey herds may possibly be related to the size of teat. All farms used the same size of teat cup liner.

(8) Of the quarters reported clinical in any one year, only 10% were known to be clinical a second time.

(9) We have no measure of the effect of treatment of all clinical cases on the incidence of infection in these herds. Also, it is not known what percentage of bacteriological cures took place, but the use of penicillin (from October 1946) did not cause a reduction in clinical cases in herds no. 4 and E during 1947 and 1948.

After omitting the clinical cases from most years in which management was considered to have influenced the number in some months only, there is found, on the average, to be a seasonal incidence (see right-hand column of Table 4) which is lowest in December and May. These results are in close agreement with those reported previously (10, 12) for clinical cases in dry and lactating cows as a whole, but not for cases in lactating cows only, where the incidence was reported (12) to be highest in January to March, even after correction for stage of lactation. The only known change in management which accompanied the low incidence in April and May was the start of spring grazing of feed rye and leys from early April. The influence of stage of lactation on the results has not been examined, but calving dates appeared to be well distributed.

Clinical symptoms. It is of some importance to know what kind of symptoms the cowmen observed and whether their reports varied greatly. In order to determine this we examined a sample of 178 consecutive reports on clinical cases in herd E, and these have been grouped in Table 5.

Table 5. *Classification of clinical symptoms reported by cowmen*

(A sample of 173 consecutive cases from farm E.)

	No clots reported		Clots and/or discoloured milk			
	Udder swollen	Udder hard and swollen	Udder swollen	Udder hard and swollen	Udder not hard or swollen	Total
Total no. of cases	36	34	54	33	16 (9 %)	173
Staphylococcus cases	20	19	34	24	9	106 (61 %)
Total	70 (40 %)		103 (60 %)			

It is interesting to find that 40% of the cases were detected before clots, if any, appeared, and that only 9% were detected in the first instance by the presence of clots or discoloured milk.

Two of the cowmen told us that if clots in the milk were the first sign to be observed they had been careless. It seems therefore, that in at least 90% of these cases inflammation was the first symptom detected. These cowmen were, however, very experienced and keen. Furthermore, none of the cases were caused by *Strep. agalactiae*, but 61% were associated with staphylococci.

The incidence of teat orifice erosions and teat sores was low. The percentage of cows with eroded teat orifices, averaged fourteen in herd E, twenty in herd 4 and fourteen in herd 3. The percentage average of cows with sores or small abrasions on their teats was thirteen in herd E, sixteen in herd 4 and thirty in herd 5.

Bacteria associated with clinical cases. The distribution of 638 reported clinical cases between the four quarters of the udder and type of organism found in the secretion from the 587 cases examined bacteriologically is shown in Table 6.

Taking the results as a whole the hind quarters were reported to be clinically affected more often than the fore quarters. This was particularly the case in the first three herds which also reported most mastitis. These results are in agreement with some previous reports (10, 23), but not with others (11, 26).

The particularly high incidence in the left hind (LH) quarters of herd E is difficult to explain, but this was also the quarter affected in two-thirds of the cases previously reported (15) as occurring in this herd. Two other reports (24, 25) also record the highest incidence in the left hind quarter. The data has not yet been fully examined, but this quarter was not the most frequently affected in every year.

The usual explanation for the belief that the hind quarters are most frequently affected is that they are more often injured and usually dirtiest. The ratio of hind quarters to front quarters in respect of both milk yield and clinical mastitis is the same. Arthur (10) reported a similar ratio for mastitis.

It was frequently observed that a succession of cows was reported clinical in the same quarter. This was true for both *Strep. dysgalactiae* and staphylococcus infections. Series of three and four cows were common, but occasionally an almost unbroken series of seven to ten cows were affected in the same quarter. The bulking of a large amount of data tends to mask these occurrences, which are very frequent in certain years.

Results of this kind may be attributed to faults in management or to contagion by more virulent strains of bacteria. Examinations made on several occasions did not reveal any faults in the teat-cup or clawpiece of the milking machine. Although there is no proof that staphylococcus mastitis is a contagious disease, Schalm & Ormsbee (27) have produced some evidence that it is contagious and our experience leads to the same conclusion. The predominance of clinical cases in the hind quarters was not confined to those caused by staphylococci. Table 6 shows that staphylococci were associated with the majority of clinical quarters and that the next most frequently isolated organism was *Strep. dysgalactiae* (Minett group II). Staphylococci predominated also in the subclinical infections shown in Tables 7 and 8. Thus 75% of the infections in 850 infected cows were associated with staphylococci and these organisms were found in 64% of the clinical cases.

These herds were under the supervision of Mr E. A. Hudson, M.R.C.V.S., who states that, in his experience, the incidence of mastitis was no more than average and that about 90% of the mastitis cases in his practice were caused by staphylococci.

It is of course not surprising that in 7·5% of cases no organism could be incriminated because only one sample was received before treatment started. There is certainly no case

Table 6. *The distribution of clinical cases between the four quarters of lactating cows and the type of organism isolated*

Farm	Data collected from	No. of times each quarter was clinical				The number of quarters containing											Total clinical quarters	Total with streptococci*		Total with staphylococci*	
						Streptococci (Minett)															
		RF	LF	RH	LH	I	II	III	S	S and I	S and II	S and III	Coliform	S and coliform	*Cory. pyogenes*	Unknown	Not tested		No.	%	No.	%
E	Oct. 1943 to Sept. 1950	40	40	57	84	0	7	25	144	0	2	5	6	0	3	13	16	221	39	19	151	74
D	Oct. 1942 to Sept. 1950	20	26	52	44	0	38	16	49	0	10	0	3	5	4	10	7	142	64	47	64	47
4	Oct. 1943 to Sept. 1950	20	20	23	33	3	12	1	59	1	3	0	2	0	0	7	8	96	20	23	63	72
5	Oct. 1943 to Sept. 1950	24	14	17	27	2	13	6	46	0	5	0	1	0	2	6	1	82	26	32	51	63
3	Oct. 1943 to Sept. 1950	6	11	5	16	5	5	3	14	1	3	0	0	0	3	1	3	38	17	49	18	51
F	Oct. 1947 to Sept. 1950	16	8	19	16	2	7	0	25	1	1	0	0	0	0	7	16	59	11	26	27	63
Total for all farms		126	119	173	220	12	82	51	337	3	24	5	12	5	12	44	51	638	177	30	374	64
Percentage of 638 quarters		38·4		61·6		—	14	9	57	—	—	—	—	—	—	7·5 of 587 examined	—	—	—	—	—	—

RF = right fore quarter. S = haemolytic staphylococci. The vast majority produced $\alpha\beta$-haemolysis on ox blood plates.
III = *Strep. uberis*, but also in this column are included Lancefield group D and some other aesculin splitting streptococci.
Unknown = no bacteria found or a very mixed flora. Most cases yielded aesculin splitting streptococci after enrichment.

* These columns contain the quarters with single and mixed infections. The percentages are based on the quarters tested, i.e. excluding clinical cases from which no samples were received.

for postulating causes other than bacterial, and it would be dangerous to make a diagnosis on the results of an enrichment method. Had diagnosis been based on enrichment

Table 7. *Udder infections in herds free (or relatively free) from* Streptococcus agalactiae

(All clinical cases treated by a veterinary surgeon. Each column refers to one herd test unless otherwise stated. Percentage figures in parenthesis.)

Farm D. Guernsey; founded in 1937; bail machine; moulded liner type 1; vacuum 14 in. 1939–40 cows with group B streptococci removed. Hand stripping until December 1949

	1943	Mar. 1944	Nov. 1944	1945	1946	1947	1948	1949	Nov. 1950
No. of cows tested	52	52	50	63	64	—	—	—	70
Cows with group B streptococci	0	0	0	0	0	—	—	—	3
* Cows with other streptococci	3	4	3	5	8	—	—	—	9
Cows with staphylococci	12 (23)	7 (13)	7 (14)	14 (22)	29 (45)	—	—	—	34 (49)
Total infected cows	14 (27)	11 (21)	10 (20)	22 (35)	34 (53)	—	—	—	40 (57)
Annual clinical cases	12	18		9	30	24	10	14	12

Moulded liner type 2 fitted in November 1949.

Farm E. Shorthorn; bail machine; stretched liner type A; vacuum 13½–15 in. 1939–41 cows with group B streptococci removed

	Sept. 1943	1944	1945	1946	1947	1948	1949	Nov. 1950	Mar. 1951	July 1951
No. of cows tested	46	39	52	44	—	—	—	57	55	56
Cows with group B streptococci	0	0	0	0	—	—	—	0	0	0
Cows with other streptococci	6	4	5	6	—	—	—	11	5	3
Cows with staphylococci	6 (13)	7 (18)	8 (15)	9 (20)	—	—	—	26 (46)	21 (38)	18 (32)
Total infected cows	12 (26)	11 (28)	13 (25)	13 (30)	—	—	—	31 (54)	23 (42)	18 (32)
Annual Clinical cases	31	14	13	15	21	57	55	47	22*	

Moulded liner type 1 fitted in February 1948. Stretched liner type A fitted in January 1950.
* In the first 11 months.

Herd 4. Shorthorn; founded November 1941; bail machine; various liners; vacuum 13½–14½ in. Hand stripping until December 1949. Stretched liner type A until April 1947.

	1945	1946	1947	Apr. 1948	Dec. 1948	Dec. 1948	1949	Feb. 1950	Nov.* 1950
No. of cows tested	—	—	—	45	49	61	59	59	55
Cows with group B streptococci	0	0	—	0	6	2	0	0	1
Cows with other streptococci	—	—	—	0	1	4	4	6	5
Cows with staphylococci	—	—	—	25 (56)	29 (59)	40 (66)	38 (64)	23 (39)	13 (24)
Total infected cows	—	—	—	25 (56)	29 (59)	40 (66)	40 (68)	28 (47)	17 (31)
Annual clinical cases	3	0	12	23			36	9	

Moulded type 1 liners March 1947. Stretched liners type A, January 1950.
* Almost a new herd composed of Guernsey cattle.

Any difference in the number of *Str. agalactiae* infections recorded in this table and Tables 1–3 results from the inclusion in the latter tables of all samples revealing the streptococcus by direct plating, whereas this table shows only those cows regarded as 'infected' with the streptococcus (see text).

methods, except in those cases where innumerable staphylococci were found, over 60% of cases would have been attributed to streptococci.

Table 8. *Udder infections in herds free (or relatively free) from* Streptococcus agalactiae

(All clinical cases treated by a veterinary surgeon. Each column refers to one herd test unless otherwise stated. Percentage figures are in parenthesis.)

Farm 3. Guernsey; founded June 1941; bail machine; moulded liner type 1; vacuum 13–15 in.

	1943	1945	1946	1947	1948	1949	Mar. 1950	Nov. 1950
No. of cows tested	25	—	55	—	—	—	61	63
Cows with group B streptocci	0	—	0	—	—	—	1	2
Cows with other streptococci	2	—	7	—	—	—	11	10
Cows with staphylococci	5 (20)	—	13 (24)	—	—	—	34 (56)	27 (43)
Total infected cows	8 (32)	—	19 (35)	—	—	—	40 (66)	31 (49)
Annual clinical cases	—	1	3	7	0	10	11	

Moulded liner type 2 fitted in December 1949. Machine stripping from December 1949.

Farm 5. Guernsey; founded August 1943; bail machine; moulded liner type 1; vacuum 13½ in.

	Mar. 1944	Nov. 1944	1945	1946	1947	1948	1949	May 1950	Nov. 1950
No. of cows tested	35	47	38	49	—	—	—	62	68
Cows with group B streptococci	0	0	0	0	—	—	—	6	7
Cows with other streptococci	0	0	1	1	—	—	—	1	8
Cows with staphylococci	2 (6)	4 (9)	6 (16)	14 (29)	—	—	—	46 (74	37 (54)
Total infected cows	3 (9)	4 (9)	7 (18)	16 (33)	—	—	—	48 (78)	40 (59)
Annual clinical cases	2		0	20	18	9	3	12	

Moulded liner type 2 fitted in November 1949. Hand stripping.

Farm F. Guernsey; founded January 1948; bail machine; moulded liner type 1; vacuum 13–15 in.

	Feb. 1948	Apr. 1948	1949	1950
No. of cows tested	30	41	—	60
Cows with group B streptococci	3	2	—	0
Cows with other streptococci	1	1	—	8
Cows with staphylococci	13 (43)	17 (41)	—	23 (38)
Total infected cows	14 (47)	19 (46)	—	28 (47)
Annual clinical cases	21		21	14

Moulded liner type 2, fitted in January 1950. Machine stripping from December 1949.

Any difference in the number of *Strep. agalactiae* infections recorded in this table and Tables 1–3 results from the inclusion in the latter tables of all samples revealing the streptococcus by direct plating, whereas this table shows only those cows regarded as 'infected' with the streptococcus.

Types of infection found at each herd test. Farm visits for the purpose of taking milk samples from all the cows were not very frequent as our main interest was clinical mastitis. The results of these herd examinations, however, show that the six herds have lately had a high incidence of staphylococcus udder infections, and that in four of the herds (D, E, 3 and 5), which were tested by direct plating methods before 1948, there has been an increase of infections caused by this organism. The results are condensed in Tables 7 and 8. The definition of 'infection' has been given on p. 16.

In herds 3, 5 and D (Tables 8 and 7), the increase in the number of staphylococcus infections accompanied an increase in the average age of the animals (Table 4). This, however, was not true of herd F (Table 8) and probably not true of herd E from 1943 to

1946. An increase of staphylococcus infections with the age of the animals has been suggested by some investigators (8, 27).

In herds 5, D and F there appeared to be no relation between the incidence of infection and the amount of clinical mastitis reported, but there was an indication of a relationship in herds 3, 4 and E. The lack of correlation between infection and clinical incidence has been noted by others in herds infected with *Strep. agalactiae* but our results may, in fact, be due to the omission of some clinical cases (see herd 5, 1946 and 1950).

When several herd examinations were made in the same year there was fair agreement between the results. A marked fall in the number of infections found is therefore probably significant. But in most cases of this type there were changes in cowshed management, and it is not possible to form sound conclusions as to the cause of the decrease. The decrease in staphylococcus and in total infections, however, which occurred in most herds in 1950, was probably the result of changes made in the milking machines late in 1949 or early in 1950. While most herds ceased hand-stripping at this time, the decrease in infections also occurred in herd 5, where hand stripping was practised.

The high percentage of staphylococcus infections found in herd 4, between 1948 and 1949, was probably associated directly with the machine used during that period. It is likely that a similar result occurred in herd E during the same 2 years. If it was connected with the machine it seems that the same machine produced an effect more rapidly in the Shorthorn than in the Guernsey breeds.

Other workers have observed a relationship between the incidence of staphylococcus mastitis and type of milking machine (28, 29).

Sensitivity to penicillin. Coagulase positive staphylococci isolated in 1950 from 276 infected quarters of cows in these six herds were examined on sheep blood agar plates for their sensitivity to penicillin and dihydrostreptomycin. Clinical cases in these herds had been treated with penicillin since 1946 and latterly in some cases with dihydrostreptomycin. Only one culture was found to be relatively resistant to penicillin, the others were sensitive to less than 0·1 unit/ml. The cultures showed no difference from the Oxford staphylococcus in their sensitivity to the other antibiotic.

CONCLUSIONS AND SUMMARY

The difficulty of keeping herds free from *Strep. agalactiae* for long periods has been demonstrated in five herds. In one sense, however, these herds were not representative of the majority of herds for they were relatively large and under the same management. Furthermore, if one herd became infected with the streptococcus, there was a real risk of the infection spreading to all herds via the hands of the relief milker, particularly if hand stripping was practised and no special precautions were taken by the relief milker.

There is no doubt that herds under the same management should take stringent precautions against the spread of infection between herds.

From a long-term point of view, the human throat carrier may be of some importance, though the abolition of hand stripping may reduce the risk from this source.

It has been shown that some herds had a very high incidence of staphylococcal infections and of clinical mastitis and it appears that the amount of mastitis in these herds was related to the type of milking machine.

Of the clinical mastitis reported from these herds, 64% of cases were associated with staphylococci and 2% with *Strep. agalactiae*. The majority of subclinical infections were

also caused by staphylococci and reached at least 70% in one herd. That these herds are not peculiar in having a high incidence of staphylococcus mastitis is supported by the experience of Mr E. A. Hudson, and by data collected over periods of 2–5 years in two other Shorthorn herds milked by the same type of machine as was used by the herds described, in 1948 and 1949.

Although *Strep. agalactiae* did not spread rapidly in these herds, except perhaps in herd 4, its spread was more rapid than that reported in certain herds practising segregation. For example, Plastridge *et al.* (31) and Cunningham *et al.* (2) give data which show that in a number of herds no new infections occurred in 12 months, although one infected cow had been retained in the herds.

In herd 3, although four cows were clinically affected with *Strep. agalactiae* in 1949, a herd examination of sixty-one cows in March 1950 revealed only one infected cow. In herd 4, no *Strep. agalactiae* infections were found in April 1948, whereas eight of sixty-one cows examined 8 months later were infected. It is improbable that treatment of clinical cases only, would have kept the incidence of infection at a very low level.

When enrichment methods, together with solid media selective for streptococci, are used for diagnostic purposes, many staphylococcus infections will undoubtedly be wrongly attributed to streptococci, particularly when only one milk sample is examined. Although this is well known, it appears to require further emphasis.

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637.125

459. THE DETERIORATION OF MILKING RUBBERS

II. THE EFFECT OF FAT

By E. R. GARDNER
Avon India Rubber Co. Ltd., Melksham, Wilts.
and N. J. BERRIDGE
National Institute for Research in Dairying, University of Reading

Discarded milking machine liners are always greasy, swollen and misshapen. All these conditions are caused by the absorption of fat and all interfere with milking efficiency. The greasiness of the surface makes the mounting of stretched liners difficult or impossible, and causes all types to slip off the cows' teats easily, the swelling of the rubber interferes with the maintenance of proper tension, and the loss of shape causes leakage of air into the vacuum line, allowing the liners to fall off the teats. This paper describes a general investigation of phenomena associated with fat absorption along the following lines:

I. The origin of the fat.
II. The effect of different kinds and conditions of fat on the amount absorbed by rubber.
III. The effect of tension in rubber on the amount of fat absorbed.
IV. The damaging effect of fat itself on rubber.
V. The re-use of old liners after the removal of absorbed fat.
VI. The effect of absorbed fat on the subsequent oxidation of rubber.

I. THE ORIGIN OF THE FAT

Preliminary experiments made some years previously had shown relatively little fat absorption by liners immersed in milk. This was confirmed by the following experiment. Samples cut from natural rubber liners were immersed for 21 days at room temperature in milk preserved by the addition of 0·1% of mercuric chloride. The milk was renewed at weekly intervals. Under these conditions the rubber increased in volume by about 2%. Under the same conditions the percentage increase in volume in contact with rape seed oil was 80–90%. Thus the absorption of fat from milk during the actual operation of milking is likely to be quite small, and the possibility of the absorption of grease from the cow's skin had to be considered. Therefore an ethereal extract of the greasiest portions of a moulded liner (including the orifice that came into contact with the udder) was subjected to a brief chemical examination. 3·5 g. of ether-soluble material, equivalent to 39% of the swollen rubber, were saponified in the usual way with alcoholic KOH. The unsaponifiable residue was weighed and examined with the results shown in Table 1. The saponification equivalent was calculated, the fatty acids in a portion of the saponified material were distilled and tested and the stearic acid content was determined by the method of Hehner & Mitchell (1). These values calculated back to saponifiable material are shown in Table 2 and compared with those for butterfat. It was concluded that about 80% of the material extracted had been butterfat, the remainder being derived from the sebaceous secretion of the cow and from the rubber itself. Further work on this subject is in progress.

Table 1. *Examination of the unsaponifiable residue from the ethereal extraction of a milking liner*

Quantity soluble in alcohol and in ether	12 % of the total extractives
Quantity not soluble in alcohol or ether	1 % of the total extractives
Average equivalent weight of soluble material (by acetylation)	223
After recovery from acetylation (hydrolysis by KOH) this was divided by fractional crystallization from alcohol into:	
(*a*) a more soluble portion showing a few cholesterol crystals	
(*b*) a less soluble portion crystallizing in scales with a melting point of 81–82° C. The weight of this material remaining after losses incidental to fractional crystallization was 13 % of the unsaponifiable residue	

Table 2. *Examination of saponifiable material from a milking liner*

	The extract	Butterfat
Saponification equivalent	248	253
Volatile fatty acids*	5·3 %	4·8–6·4 %(3)
Stearic acid	Undetectable	0·5 %†
Non-volatile fatty acids	Approx. 80 %	90 %(3)

* Soluble only, calculated as butyric.
† Leathes & Raper(2). By another method 8–12 % of stearic acid has been found in milk fat (Hilditch(3)).

II. THE EFFECT OF DIFFERENT KINDS AND CONDITIONS OF FAT ON THE AMOUNT ABSORBED BY RUBBER

From the experiments of § 1 it appeared that although little fat is picked up by liners immersed in milk, most of the fat present is derived from milk and, as there is little information available relating to the behaviour of rubber in oil-water emulsions, experiments were made in these media. Different emulsions were made as shown in Table 3. The oil content of each emulsion was approximately equal to the fat content of milk. Except for slight differences, all the emulsions were prepared by stirring the oil with the emulsifying agent, adding about 400 ml. of water with constant stirring and finally adding the thick emulsion so obtained to the remainder of the water. During the period of immersion of the rubber samples, the temperature of the emulsions was maintained at 35° C. and they were slowly stirred. No measurements of particle size were made and no account was taken of any difference in swelling rate due to variations in particle size. The rubber samples, 2×1 in. in area, were cut from $\frac{1}{16}$ in. sheets of natural rubber milk liner compound prepared in the laboratory. The period of immersion was 7 days for *A*, *B* and *C* and 5 days for *D*. The pH of emulsion *A* was 6–7 and that of *B*, *C* and *D* 7–8.

The figures show the great influence of the emulsifying agent on the rate of swelling of the rubber. In another experiment, an emulsion of the same composition as *C* caused an

Table 3. *The swelling of rubber in different emulsions*

Compositions of emulsions (grammes in 6 l. of water)

	A	*B*	*C*	*D*
Raw linseed oil	200	200	240	240
'Petromore Pale A'	40	40	—	—
Casein	—	100	—	—
Borax	—	35	—	—
Sodium oleate	—	—	10	—
Ammonium linoleate	—	—	—	8·5
Increase in volume of rubber samples (as % of original volume)	13	6	40	89

increase in volume of 13·9% in 7 days at 35° C., but another portion of the same emulsion containing in addition 0·83% caustic soda (final pH 10) caused a swelling of only 0·5%. (The difference between the two emulsions *C*, 13·9% against 40%, may have been due to different degrees of emulsification of the oil.) Thus caustic soda prevents the take-up of oil. This is in agreement with experiments described below on the use of caustic soda solution for removing fat from liners.

A further experiment was designed to approach the conditions to which liners are subjected during use. Several batches of rings from liners of the stretched type (probably G.R.S.) were allowed to swell in fat under the following conditions:

First batch: no treatment.

Second batch: covered with dry butterfat and heated to between 100 and 120° C. on thirteen occasions spread over 44 days.

Third batch: covered with butterfat containing only enough distilled water to allow of its being boiled, not enough to prevent contact between rubber and fat, and boiled on sixteen occasions spread over 44 days. The water was replenished as necessary.

Fourth batch: covered with extract of cow hair, prepared by ethereal extraction and evaporation, and heated twenty-one times during 44 days, i.e. approximately every other day. The fat seemed to form acrolein rather readily and therefore a little water was added occasionally to prevent overheating. Temperatures from 94 to 105° C. were recorded.

Fifth batch: covered with cream diluted with half a volume of water and half a volume of milk and boiled on twenty-one occasions during 44 days, i.e. approximately every other day, but daily for the first 3 days for sterility.

Sixth batch: covered with thick cream, and autoclaved twice (120° C. 15 min.), once at the start, again on the third day.

The amount of fat absorbed under each of these conditions is shown in Table 4, from which it is clear that emulsified fat is less readily absorbed. In the cream samples the concentration of fat was much greater than in milk and the artificial emulsion experiments. Moreover, partial breaking of the emulsion probably occurred. These two factors would account for the relatively great absorption of fat from the two cream samples.

Table 4. *The swelling and elasticity of rubber*

Ring no.	Treatment (see text)	Fat content % (substances extractable by ether)	Modulus of elasticity at 5 kg./sq.cm. (see text, § IV) (*a*) Using actual area of cross-section	(*b*) Using fat-free area of cross-section
1*a*	None (new)	7·3	18	17
2*a*	Dry butterfat	49·1	10	14
3*a*	Wet butterfat	48·7	8	10
4*a*	Wet cow sebum	37·2	9	12
5*a*	Cream boiled repeatedly	19·2	12	14
6*a*	Cream autoclaved twice	32·2	10	13
1*b*	As 1*a*, extracted	—	19	—
2*b*	As 2*a*, then fat extracted	—	24	—
3*b*	As 3*a*, then fat extracted	—	19	—
4*b*	As 4*a*, then fat extracted	—	19	—
5*b*	As 5*a*, then fat extracted	—	19	—
6*b*	As 6*a*, then fat extracted	—	21	—

These results emphasize that the fat must be deposited on the rubber from the emulsion before absorption can occur. The readiness with which this happens depends on

the emulsifying agent. Fortunately, the emulsion in milk is particularly stable. Otherwise the use of natural rubber in milking machines would hardly be possible.

III. THE EFFECT OF TENSION IN RUBBER ON THE AMOUNT OF FAT ABSORBED

In view of the fact that many rubber teat cup liners are under tension during use, it is of interest to find how various degrees of stretch affect the rate of fat absorption. It has been predicted from thermodynamic considerations that stretch increases the rate of swelling (Flory & Rehner (4), Treloar (5)). These authors checked their theory by determining the rates of swelling of stretched rubber in aromatic hydrocarbon solvents. The swelling in these solvents is very rapid, and it was considered desirable to conduct similar experiments with oils and emulsions in which the swelling is much slower. It was found over the range of stretch used in the experiments, i.e. 0–75%, that increase in stretch increased the rate of swelling in both oils and emulsions.

IV. THE DAMAGING EFFECT OF FAT ITSELF ON RUBBER

The lubricating and swelling effects of fat need no confirmation, although they were confirmed incidentally later in experiments on the removal of fat from discarded liners. Attention was therefore concentrated on the modulus of elasticity, and it was confirmed that this property was smaller in discarded liners than in new ones. When rings from discarded liners were heated with caustic soda as suggested by Moir (6) to remove absorbed fat the modulus was increased somewhat, but did not reach the level for new rubber. It appeared that some fat remained after Moir's treatment when applied to this particular kind of rubber (G.R.S.). Therefore further experiments were done using extraction with ether.

Four adjacent rings *A–D*, 3–4 mm. wide, were cut from a baggy discarded liner of the stretched type. Two of them were thoroughly extracted with ether and allowed to dry. Their elasticities were then roughly compared (see Table 5).

Table 5. *Values of a modulus of elasticity, $Tl_o/(l_t-l_o)$, for various rubber rings*

(l_t = length (between marks) under tension; l_o = original length (no tension); T = tension in kg./sq.cm. of the cross-section while under tension.)

Source of rings...	Old liners					Old liners				New liners	
Treatment ...	None					Extracted with ether				None	
Designation of rings ...	*B*	*C*	12	16	20	*A*	*D*	14	18	α	β
Fat content (% of fatty weight)	—	—	34	27	13	—	—	—	—	—	—
Limits of T:											
0·7– 1·4	17	11	16	14	—	24	53	14	23	11	18
3·4– 5·0	10	9	12	15	20	22	28	25	28	18	—
7·0–12	10	10	13	15	19	18	20	24	26	18	18
17 –22	13	—	15	16	20	—	—	24	25	18	18
25 –35	16	14	16	18	22	20	21	25	26	21	—
41 –53	—	—	—	20	25	26	26	26	29	24	24
3·4–22 (means)	11	10	13	15	20	20	24	24	26	18	18

More results were obtained on rubber from a second greasy liner. It was sliced into twenty-two rings (numbered consecutively) giving a width of about 7 mm. per ring. Numbers 14 and 18 were extracted with ether and then compared with numbers 12, 16

and 20, and with rings from a new liner (which was probably of a different kind of rubber). The figures are presented in Table 5. They do not present a true Young's modulus as they increase with tension, but they are roughly constant over a narrow range. Therefore only the range of tension at each level is recorded in place of all the individual values, and for a rough comparison the average over a wider range has been used. The errors are obviously large, but not large enough to obscure the great differences between the rubbers. The higher values for slice 20 are due to its lower fat content. The new rubber, α and β, was a rather soft kind.

The presence of fat in the rubber increases its volume. The cross-sectional area is then greater than that of fat-free rubber and the apparent tension, calculated by dividing the weight by this area, is correspondingly smaller. Since the fat does not contribute to the strength of the mixture the true tension in the rubber itself is greater than that shown. The modulus of elasticity will therefore be greater when allowance is made for this fact, and the greasy rubber will appear stiffer. The relative importance of this factor may be judged from those figures of Table 4 which have been calculated by both methods. The main conclusion, that the rubber is considerably weakened by the presence of fat, is not invalidated.

It was next of interest to confirm these results by allowing new rubber to absorb fat and to determine the effect of differences in the kind and condition of fat. The treatment of the rubber has already been described in § II. Rings from each batch were tested for modulus of elasticity. Others were thoroughly extracted with ether and also tested. In Table 4 are given the amounts of fat absorbed under the various conditions and the moduli at a load of 5 kg./(sq.cm. under tension). Roughly speaking, modulus is inversely proportional to the amount of fat absorbed, sebum being no more effective than butterfat, and when the fat has been extracted the modulus is greater than that for new rubber because of the removal of softeners. No. 2 is an exception; its greater stiffness may be due to the higher temperatures used, causing a little further vulcanization.

V. THE RE-USE OF OLD LINERS AFTER THE REMOVAL OF ABSORBED FAT

Since the softening effect of absorbed fat was now demonstrated, attempts were made to put old liners back into use by removing the fat. A set of very soft greasy liners of the stretched type, which had not been cut, were extracted by soaking in repeated changes of ether. When the extract was only slightly coloured, the liners, now greatly swollen, were removed and the ether allowed to evaporate. The liners were then normal in size, shape and elasticity. They were used again on the farm for a further 5 weeks, i.e. for half the time new liners would be expected to last.

Because of the inconvenience of solvent extraction, cold caustic soda was tried for removing fat from used liners. Saponification must have occurred slowly at room temperature for, after several weeks immersion in 10% caustic soda, liners became covered with a layer of soap. When the soap had been scraped and washed off the rubber appeared as new.

Caustic soda was then tried on the farm. Two sets of liners per cluster were used and changed once each fortnight, the set not in use being left to soak in $7\frac{1}{2}$% caustic soda. (As part of another experiment the set in use were kept full, between milkings, with 0.5% caustic soda.) The useful life of the liners was prolonged by approximately 50%, in agreement with the ether extraction experiment.

Old liners from which the fat has been removed are therefore approximately half as good as new. A possible explanation of why they are not better than this came out of the experiments on oxidation, which are reported below.

VI. THE EFFECT OF ABSORBED FAT ON THE SUBSEQUENT OXIDATION OF RUBBER

To investigate further the reason why used liners, from which fat has been removed, do not last as long as new ones, samples of rubber were swollen with butterfat for 2 days at room temperature, whereby the rubber absorbed roughly 30% of its weight of fat, and then stored at room temperature for 5 months. Two rubber compounds were used, they were identical except for the presence of an antioxidant in one of them. Each compound was vulcanized for 30 to 45 min. at 280° F. Both fresh fat and a sample oxidized by exposure to air and sunlight were used as swelling media, and samples were stored both in daylight and in darkness. At the end of the storage period the rubber samples were extracted with acetone to remove the fat and the tensile strength and elongation at break were determined. These tests, which reflect the ordinary wearing qualities of rubber, were made by cutting dumbbell-shaped samples and stretching to break under standard conditions on a dynamometer. The strength at break was expressed as kg./sq.cm., using for the computation the area of cross-section before stretching. The results are shown in Table 6.

Table 6. *Strength of rubber after oxidation in the swollen condition at room temperature and subsequent removal of the fat*

Rubber mix ...	R 189 A (with antioxidant)				R 189 B (without antioxidant)			
Conditions of storage ...	Exposed to light		Kept in dark		Exposed to light		Kept in dark	
Conditions of cure and swelling	Tensile strength (kg./sq.cm.)	Elongation at break (%)	Tensile strength (kg./sq.cm.)	Elongation at break (%)	Tensile strength (kg./sq.cm.)	Elongation at break (%)	Tensile strength (kg./sq.cm.)	Elongation at break (%)
Cure 30 min./280° F.:								
Swollen with unoxidized fat	227	570	256	590	140	500	172	550
Swollen with oxidized fat	234	570	189	540	169	500	226	560
Cure 45 min./280° F.:								
Swollen with unoxidized fat	213	570	179	550	163	530	157	500
Swollen with oxidized fat	187	570	199	560	134	520	164	530

The tensile strength of the rubber mixes immediately after vulcanization is about 260 kg./sq.cm., the value varying very little between the two mixes and the two cures. The decrease in tensile strength of the rubber on standing for 5 months in its normal state would not be expected to be greater than 5%. It will be seen that the breakdown in the swollen state is much more rapid. In order to investigate the effect of the various factors, the tensile strength figures were submitted to analysis of variance with the result shown in Table 7.

The factors which are obviously insignificant were pooled with the residual sum of squares and the significance of the other factors was tested using this new value. The

$A \times C$ and $D \times A \times B$ interactions were only just significant at the 5% level and it therefore appears that only between mixes and between cures variances are truly significant, the former at the 0·1% level and the latter at the 1% level. If it is assumed that the tensile strengths of all the rubber samples were approximately equal before swelling and ageing, it may be concluded that the inclusion of an antioxidant and the maintenance of vulcanization times at the minimum, assist in minimizing the deterioration. The state of oxidation of the fat and the presence or absence of light appear to be without effect.

Table 7. *Analysis of variance of tensile strength figures from Table* 6.

(The degrees of freedom are 1 for each factor.)

	Sums of squares
Between mixes (A)	8,055
Between cures (B)	2,943
Between exposure conditions (C)	352
Between states of oxidation of fat (D)	2
$A \times B$	95
$B \times C$	315
$C \times D$	67
$D \times A$	1,008
$A \times C$	1,425
$B \times D$	162
$A \times B \times C$	218
$B \times C \times D$	1,074
$C \times D \times A$	496
$D \times A \times B$	1,581
Residual	740
Total	18,533

The deterioration of rubber under normal conditions, or when subjected to heat in the presence of air, is due to oxidation proceeding as a chain reaction (7). The deterioration in the swollen state occurs more rapidly, and this is no doubt due to the conditions then being more favourable to the propagation of the chain reaction.

Some further work on the deterioration was carried out using dumbbell-shaped samples cut from rubber sheets vulcanized in the laboratory. Two milk liner rubber compounds were used, R 487 A with no antioxidant and R 487 B with a rubber antioxidant. The tensile strength, the stress under a stretch of 500% and the elongation at break were measured for one set of samples before ageing, and for another set after ageing in air at 70° C. for 7 days. A further set of samples was swollen in rape oil for 5 days at 70° C., the volume increase being approximately 90% under these conditions. After removal from the oil, the samples were aged for only 1 day in air at 70° C. and then extracted with acetone until all the oil had been removed. After the acetone had been allowed to evaporate the tensile properties were determined. The results are shown in Table 8.

Normally when rubber is extracted with acetone the tensile strength and stiffness increase. It is obvious that under hot conditions the deterioration in the swollen state is dramatic. It seems, therefore, that deterioration of milker rubber in use is at least partly due to the enhanced rate of oxidation when swollen with fat. This would particularly be the case where heat sterilization is used.

Used liners which have been stored for some time occasionally develop patches where the rubber loses most of its elasticity, becoming a plastic or semi-solid. These experiments suggest that this degeneration is due to oxidation in the swollen condition.

Table 8. *Strength of rubber after oxidation in the swollen condition accelerated by heat, and subsequent removal of the fat*

Treatment	Compound	Vulcanization time at 298° F. (min.)	Tensile strength (kg./sq.cm.)	Stress at 500 % elongation (kg./sq.cm.)	Elongation at break (%)
None	R487A	15	254	111	680
		30	258	135	700
None	R487B	15	259	116	640
		30	253	146	660
None	R487A	15	282	145	660
		30	250	156	630
Aged 7 days	R487B	15	266	166	610
		30	228	166	590
Swollen 5 days	R487A	15	77	65	530
		30	57	*	440
Aged 1 day and extracted	R487B	15	124	97	520
		30	130	*	490

* Samples broke before 500 % elongation

CONCLUSIONS AND SUMMARY

The presence of fat in milking machine liners is probably the chief cause of their deterioration. The primary effect of the fat is to cause softening and swelling of the rubber and greasiness of its surface; the secondary effect is to increase greatly the rate of oxidation. The primary effect may be reversed by removing the fat by solvent extraction or prolonged treatment with cold caustic soda; the secondary effect is irreversible.

Although the fat is derived chiefly from milk, rubber absorbs fat only slowly from liquid milk. Thus it is likely that most of the absorption is from milk residues remaining on liners between milkings, under conditions which favour the breaking of the natural emulsion. Such conditions would include drying, souring or other bacterial activity, and the presence of traces of detergents which act as protein denaturing agents.

Thus, if the widely recommended technique of cold or tepid rinsing is properly practised (8), fat absorption is kept to a minimum, and the life of liners will be prolonged. Under such conditions the absorption of fat from the cow's skin will be more important and methods of removal, or of prevention, such as the use of fat-resistant rubber may be advantageous.

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460. STUDIES OF THE SECRETION OF MILK OF LOW FAT CONTENT BY COWS ON DIETS LOW IN HAY AND HIGH IN CONCENTRATES

I. THE EFFECT OF VARIATIONS IN THE AMOUNT OF HAY

By C. C. BALCH, D. A. BALCH, S. BARTLETT, C. P. COX and S. J. ROWLAND
National Institute for Research in Dairying, University of Reading

(With 3 Figures)

The fat content of milk has usually been considered to be unaffected by changes in the non-fatty components of the diet. However, Powell (1, 2, 3) reported experiments in which cows, mainly Holsteins, were given diets of concentrates and 6 lb., or less, of hay daily for whole lactations, and found that these diets markedly depressed the milk fat content. A return to more normal diets, even after one or more lactations on the diets low in hay, restored the milk fat content to its original level. Short-term experiments conducted by Loosli, Lucas & Maynard (4) showed that Holstein cows receiving 5 lb. or less of hay daily produced less milk, of lower fat percentage, than cows fed normally, although the concentrate portions of the diets were adjusted so that both diets supplied equal amounts of energy. In a preliminary announcement Stoddard, Allen & Peterson (5) claim to have shown that when cows received concentrates *ad lib.* with less than 6 lb. hay daily, there was a very marked decrease in the fat content of the milk. It was further claimed that this decrease in milk fat content could be prevented by the administration of acetic acid, but not of propionic acid, through a stomach tube into the reticulo-rumen. The experiments of Powell, Loosli *et al.* and Stoddard *et al.* were all conducted in the United States under conditions of indoor feeding.

In England unusually low fat contents in milk have occurred from time to time over a number of years following the change from stall feeding to spring grazing. McClymont & Paxton (6), in New South Wales, first suggested that this type of depressed fat content was also due to a shortage of roughage in the diet. In the experiment of McClymont & Paxton a group of six Ayrshire cows was allowed to graze young leafy oats while receiving dry concentrates in addition. The mean milk fat content rapidly fell from about 4·3 to 2·6%, although in a similar control group, fed in exactly the same way but also receiving 20 lb. daily of a roughage (Saccaline, a variety of sweet sorghum), the milk fat content was maintained at about 4·0%. More recently, McClymont (7) has described his experiences of the various grazing conditions which have been found to reduce the fat content of milk in Australia. Most of the diets to which he refers appear to have been low in fibre and high in readily digestible carbohydrate and protein.

In England, during the course of advisory work, instances have been encountered of herd milk of unsatisfactory fat content during the winter stall-feeding period which were apparently due to the feeding of inadequate roughage to the cows. This paper describes a successful attempt to depress the fat content of milk experimentally under such conditions.

METHOD

Twelve Shorthorn cows from the Institute herd were grouped in blocks of three so that animals within blocks had calved at about the same time. Treatments were allocated at random to animals within blocks, there being one animal on each treatment in each block. The experiment lasted 11 weeks with the following feeding programme.

Period I (preliminary control), weeks 1–3. All the cows received the same daily diet consisting of 18 lb. hay, 30 lb. mangolds, and a concentrate mixture balanced for milk production (mixture E) at the rate of 4 lb. per 10 lb. milk produced. The amount of hay was adjusted slightly according to the weight of the cows above or below 1100 lb., and for poor appetite, but varied only from 17 to 21 lb.

Period II (experimental treatment), weeks 4–7. For all the cows the rationing of mangolds and concentrates for milk production continued unchanged. The control group (group A) of four cows continued to receive hay at the level given in period I, but in the other two groups of cows the daily amounts of hay were reduced, in group B to 6 lb. and in group C to 2 lb., and 9 lb. of a concentrate mixture low in protein and fibre (mixture G) were given instead. The change in the diet of group B was completed in 1 week, but the change to the more extreme treatment (group C) took 10 days. All the cows were therefore well established on the experimental treatment for the last 2 weeks of period II.

Period III (final control), weeks 8–11. All the cows were fed the same amounts of hay and mangolds as in period I, and concentrates (mixture E) were fed according to yield of milk. The change to these diets from the diets low in hay was completed in 1 week.

Foods. The hay consisted mainly of mature perennial ryegrass, and the mangolds were of the Yellow Globe variety. The constituents of the concentrate mixtures are given in Table 1. All the foods were weighed and sampled and determinations of dry-matter content were made daily. The dry samples of food were bulked and ground, mixed, and analysed at the end of each period. The mean composition of the foods is given in Table 2; the conventional determinations were made by the standard methods and cellulose was determined by the method of Norman & Jenkins (8).

Management. The cows were housed in a modern cowshed with mangers adapted to prevent loss of food. The only bedding provided was a small amount of resinous sawdust which the cows did not eat. All the foods were given daily in two equal portions; the

Table 1. *The concentrate mixtures*

	Parts by weight	
Food	Mixture E	Mixture G
Decorticated groundnut cake	1	—
Dried brewers grains	—	1
Dried sugar-beet pulp	—	1
Fine wheat feed	2	2
Flaked maize	4	6

Table 2. *Composition of hay, mangolds and concentrate mixtures* (%)

Food	Dry matter	Crude protein	Ether extract	Nitrogen-free extract	Crude fibre	Cellulose	Ash
Hay	84·21	6·17	1·49	46·22	25·20	38·73	5·14
Mangolds	9·29	1·73	0·07	3·90	0·80	0·96	2·79
Concentrate mixture E	86·74	16·16	3·17	58·94	4·52	5·93	3·96
Concentrate mixture G	85·90	10·63	3·66	62·28	6·37	8·54	2·96

concentrate mixture E was given during milking, while the hay, mangolds and concentrate mixture G was given immediately after milking. For about 6 hr. daily the cows were allowed exercise on bare ground and they were milked twice daily by machine. The cows were weighed at the start of the experiment and at the end of each period.

Milk. Composite evening/morning samples of the milk of each cow were taken on 3 days spread over each week. The fat content of the samples was determined by the Gerber method (9). The total solids content of the milk was determined gravimetrically (10) on one of the composite samples each week.

A larger sample of milk was taken on one day in each week, except week 1, for an investigation of the composition of the butterfat. On this day three pints of milk were retained from the milk of each cow at the evening milking and five pints at the next morning milking. The total daily samples from the cows on each treatment were bulked (4 gallons) and the cream was separated. The creams were churned and the butters stored at $-25°$ C. until required for analysis. Each butter was then melted, the serum removed and the butterfat filtered. The Reichert values, iodine values and saponification values of the butterfats were determined by the British Standard Methods (11), and the softening points were measured by Barnicoat's modification of the falling-ball method (12).

Statistical analysis. Multiple regression analysis was used for the results of the period of full experimental treatment, that is, for weeks 6 and 7 combined. The auxiliary variables were the mean yield in the pre-treatment standardization period of three weeks and the mean yield in the last 2 weeks of the post-treatment standardization period. For the other weeks an analysis of covariance was used taking as the auxiliary variable a weighted mean of yields in the standardization periods. For statistical analysis observations on milk fat percentage and yield of milk fat were transformed by the square root and logarithmic transformations respectively.

RESULTS

Food intake. The mean daily intake of dry matter, crude protein, ether extract, nitrogen-free extract, crude fibre and cellulose by cows in the three groups is given in Fig. 1. In arriving at these figures small amounts of uneaten hay and concentrates have been deducted.

Milk yield. The mean daily yields of milk by individual cows in each week of the experiment are given in Table 3, and the mean daily yield of each group has been used to plot Fig. 2. During the experimental period there was a distinct tendency for cows in group B (6 lb. of hay) to produce more milk and those in group C (2 lb. of hay) to produce less milk as a result of the treatment. In the weeks of full experimental treatment (weeks 6 and 7) the yield of milk by cows in Group B was greater than that of cows in the control group, the difference approaching statistical significance ($P \doteqdot 0{\cdot}055$). The difference between groups A and C was not significant ($P \doteqdot 0{\cdot}19$), while the difference between groups B and C was highly significant ($P \doteqdot 0{\cdot}009$). At the end of experimental feeding the yields rapidly returned to their pre-treatment rate of decline, and even in week 8 there was no longer any significant difference between the mean yields.

Fat content of the milk. Full details of the fat content of the daily samples are given in Table 4, and the group weekly mean values are plotted in Fig. 2. Treatment effects were discernible as a marked fall even while the diets were being changed and by weeks 6 and 7, when the animals had become established on the full treatment, the drop in the fat content

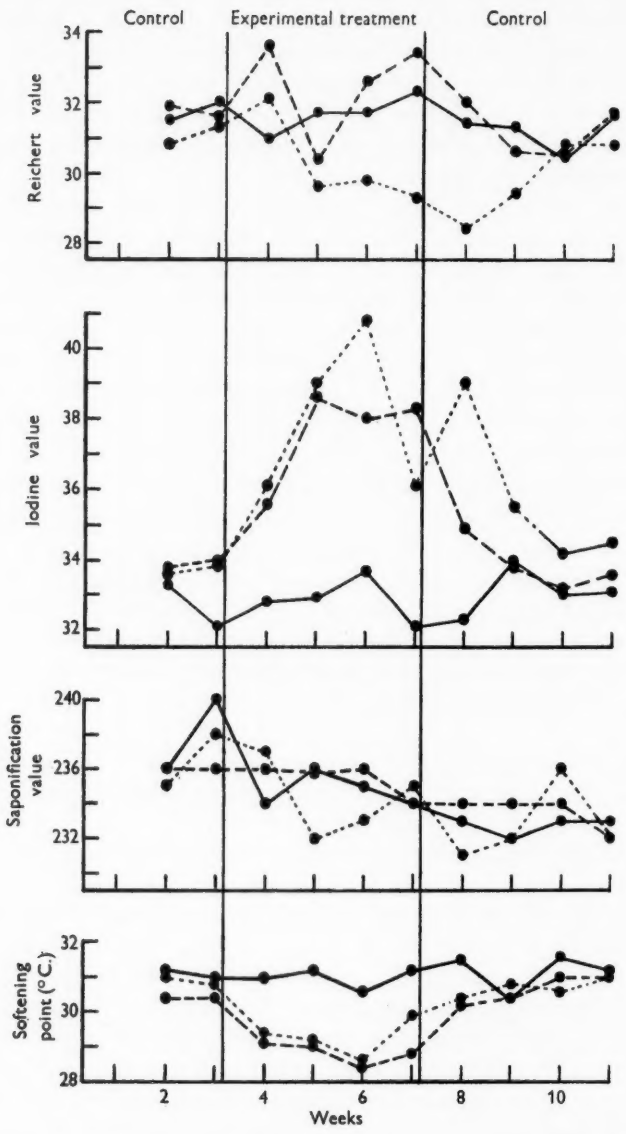

Fig. 1. The mean daily intake of dry matter, crude protein, ether extract, nitrogen-free extract, crude fibre and cellulose by groups of cows receiving daily diet A (about 18 lb. of hay), ●——●, diet B (6 lb. of hay), ●– – –●, and diet C (2 lb. of hay), ●····●, in the period of experimental treatment. During the control periods all groups received 18 lb. of hay daily.

of the milk from cows in group B (6 lb. of hay) was significant at the 5% level and that of group C (2 lb. of hay) at the 2% level. The differences between means adjusted for their slight initial differences were:

Treatment comparison	Mean difference in milk fat (%)	P*
$A - B$	0·49	0·02
$A - C$	0·59	0·01
$B - C$	0·10	0·45

* P is the probability that a difference as large as, or larger than, that observed would have arisen by chance from a normal population for which the mean difference between A, B or C was zero.

Table 3. *The mean daily yields of milk and of milk fat by cows given diets low in hay and high in concentrates*

		Group A									
		Mean daily yield of milk (lb.)					Mean daily yield of milk fat (lb.)				
Week	Diet	Cow 1	Cow 2	Cow 3	Cow 4	Group mean	Cow 1	Cow 2	Cow 3	Cow 4	Group mean
1	A (control)	30·1	41·6	26·9	43·2	35·4	1·15	1·96	1·08	1·56	1·44
2	A (control)	28·8	45·5	24·7	42·4	35·3	1·09	1·45	1·04	1·45	1·26
3	A (control)	27·9	43·9	23·4	42·6	34·4	1·14	1·53	1·03	1·45	1·29
4	A (control)	27·3	45·0	23·6	41·2	34·3	1·08	1·72	0·99	1·39	1·30
5	A (control)	26·4	45·6	23·5	40·3	34·0	0·97	1·64	1·03	1·47	1·28
6	A (control)	25·4	45·0	23·2	39·1	33·2	0·95	1·59	0·99	1·36	1·22
7	A (control)	24·2	43·5	21·6	37·5	31·7	0·94	1·56	0·92	1·31	1·18
8	A (control)	22·5	42·6	20·7	36·9	30·7	0·84	1·49	0·90	1·31	1·13
9	A (control)	21·8	40·6	21·0	35·8	29·8	0·81	1·39	0·92	1·30	1·10
10	A (control)	20·0	40·0	20·6	35·0	28·9	0·78	1·41	0·91	1·19	1·07
11	A (control)	21·2	39·7	20·6	33·9	28·8	0·82	1·50	0·82	1·18	1·08
		Group B									
		Mean daily yield of milk (lb.)					Mean daily yield of milk fat (lb.)				
Week	Diet	Cow 5	Cow 6	Cow 7	Cow 8	Group mean	Cow 5	Cow 6	Cow 7	Cow 8	Group mean
1	A (control)	34·9	31·7	30·3	35·5	33·1	1·46	1·27	1·13	1·13	1·25
2	A (control)	34·7	30·1	30·0	34·5	32·3	1·24	1·28	1·09	1·36	1·24
3	A (control)	33·1	28·3	28·4	31·2	30·3	1·27	1·05	1·04	1·26	1·16
4	Change	37·2	30·4	28·5	25·9	30·5	1·33	1·12	1·03	1·00	1·12
5	B (6 lb. hay)	38·4	32·6	27·8	28·3	31·8	1·31	1·05	0·68	0·73	0·94
6	B (6 lb. hay)	38·1	30·0	27·4	27·6	30·8	1·30	1·04	0·81	0·88	1·01
7	B (6 lb. hay)	37·4	29·5	27·0	26·4	30·1	1·15	1·00	0·94	0·86	0·98
8	A (control)	34·1	27·8	25·2	23·3	27·6	1·21	1·09	0·87	0·84	1·00
9	A (control)	31·5	24·1	24·6	21·4	25·4	1·08	0·82	0·84	0·82	0·89
10	A (control)	29·9	22·1	24·8	21·2	24·5	1·06	0·88	0·91	0·77	0·90
11	A (control)	30·8	22·1	24·5	19·1	24·1	1·15	0·90	0·94	0·71	0·93
		Group C									
		Mean daily yield of milk (lb.)					Mean daily yield of milk fat (lb.)				
Week	Diet	Cow 9	Cow 10	Cow 11	Cow 12	Group mean	Cow 9	Cow 10	Cow 11	Cow 12	Group mean
1	A (control)	27·6	35·9	32·1	30·3	31·5	1·00	1·31	1·28	1·24	1·21
2	A (control)	26·5	33·9	28·9	31·5	30·2	0·97	1·11	1·22	1·21	1·13
3	A (control)	25·0	32·4	27·0	32·1	29·1	0·96	1·11	1·05	1·17	1·07
4	Change	26·4	32·3	25·6	31·0	28·8	0·98	1·07	1·02	1·14	1·05
5	Change	24·0	29·9	25·9	33·1	28·2	0·65	0·96	0·93	1·11	0·91
6	C (2 lb. hay)	22·3	26·9	21·4	29·4	25·0	0·63	0·82	0·72	0·92	0·77
7	C (2 lb. hay)	21·2	24·7	18·9	26·9	22·9	0·59	0·79	0·68	0·94	0·75
8	A (control)	20·7	21·9	19·2	29·1	22·7	0·60	0·70	0·73	0·86	0·72
9	A (control)	20·2	21·1	18·6	27·9	22·0	0·73	0·74	0·70	1·02	0·80
10	A (control)	19·9	22·0	17·4	28·0	21·8	0·80	0·75	0·67	1·06	0·82
11	A (control)	19·6	21·2	17·5	27·8	21·5	0·74	0·75	0·66	1·11	0·81

In groups A–C the unadjusted fat percentages were 3·79, 3·29 and 3·18 respectively, the corresponding adjusted percentages being 3·77, 3·28 and 3·18.

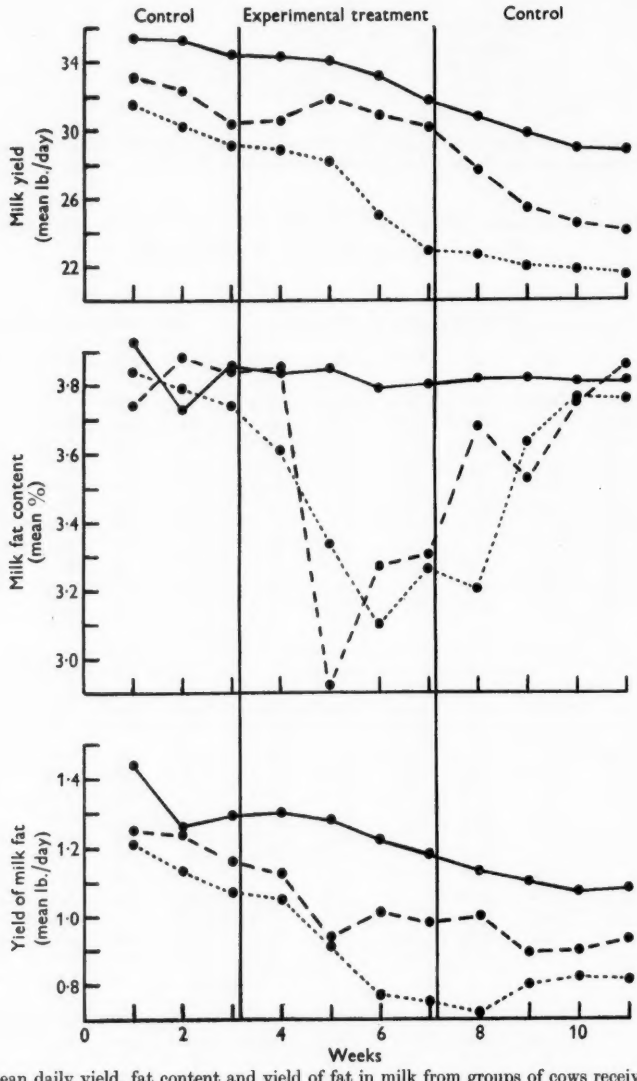

Fig. 2. The mean daily yield, fat content and yield of fat in milk from groups of cows receiving daily diet A (about 18 lb. of hay), ●——●, diet B (6 lb. of hay), ●– – –●, and diet C (2 lb. of hay), ●····●, in the period of experimental treatment. During the control period all groups received 18 lb. of hay daily.

Following the return to the control diets there was a rapid recovery to fat levels similar to those recorded in the initial control period. Even in the first week of the final period there was no significant difference between milk fat percentages in the three groups.

Yield of milk fat. The mean daily yields of milk fat by individual cows in each week of

the experiment are given in Table 3, the weekly mean values for cows in each of the treatment groups are plotted in Fig. 2. During the experimental period the increase in milk yield by cows in group B (6 lb. of hay) compensated for the decreased milk fat content and resulted only in a very small, and insignificant ($P \doteqdot 0{\cdot}34$) net fall in weight of milk fat produced. However, in group C (2 lb. of hay) the experimental treatment produced a highly significant ($P \doteqdot 0{\cdot}011$) decrease in the yield of milk fat. In weeks 6 and 7, cows in groups A–C produced daily 1·20, 0·99 and 0·76 lb. of fat per cow respectively; when equalized for initial differences in yield this amounted to 1·06, 1·00 and 0·86 lb. in the respective groups. The difference in yield of fat rapidly disappeared when the diets were changed, and by week 9 there was little difference between the values for the three groups.

Composition of butter fat. Weekly results for Reichert value, iodine value, saponification value and softening point in the group bulked samples are given in Fig. 3. There was a distinct fall in the Reichert value of butter from cows in group C (2 lb. of hay) when the diet low in hay was given, but this fall was not present in values from group B (6 lb. of hay). However, the limited analysis possible did not show that this fall was significant. In both groups the experimental treatment caused a spectacular increase in the iodine value, a change which is naturally also reflected by a fall in the softening point. These changes were statistically significant ($P \doteqdot$ approximately 0·03). The treatment apparently caused no change in the saponification value.

Solids-not-fat content of the milk. The level of solids-not-fat in the milk was not affected by the dietary changes (Table 5).

Weight of cows. The weights of the cows are given in Table 4. During the preliminary control period the mean weight of cows rose 39, 18 and 35 lb. in groups A–C respectively. In the 28 days of experimental feeding the control group (A) gained on the average a further 7 lb., while groups B and C lost 48 and 68 lb. respectively. In the final control period the average weight of group A fell 16 lb., while group B more than regained the weight lost in the experimental period and group C weighed the same as at the start of the experiment. The appearance of the cows indicated that the fluctuations in weight when the diets low in hay were given represented nothing more serious than an emptying of the digestive tract due to an intake of highly digestible food. Fluid faeces were produced by the cows receiving diets low in hay amounting initially almost to scouring; since it has usually been supposed that fluid faeces denote a rapid rate of passage of food residues through the intestine, it is likely that the diets low in hay caused a rapid emptying of at least some portions of the digestive tract.

DISCUSSION

In this experiment diets were given which were low in hay and high in concentrates as compared with normal practice, but they were nevertheless, and especially diet B (6 lb. of hay), similar to diets which are being given on some farms in Great Britain. Under normal cowshed conditions these diets caused substantial falls in the fat content of the milk. When allowance is made for very small initial differences between the three groups of four cows, the mean fat content of the milk produced in the 14 days of full treatment was: group A (control, 18 lb. of hay), 3·8%; group B (6 lb. hay), 3·3%; group C (2 lb. hay), 3·2%. Such falls would cause considerable concern to practical farmers, and particularly in countries where the payment for the milk depended on its content of fat. The extent of the fall produced by diet B decreased as the experimental

period advanced, but even its marked early low level had little effect on the total yield of milk fat because there was a simultaneous rise in the yield of milk. When only 2 lb. of hay

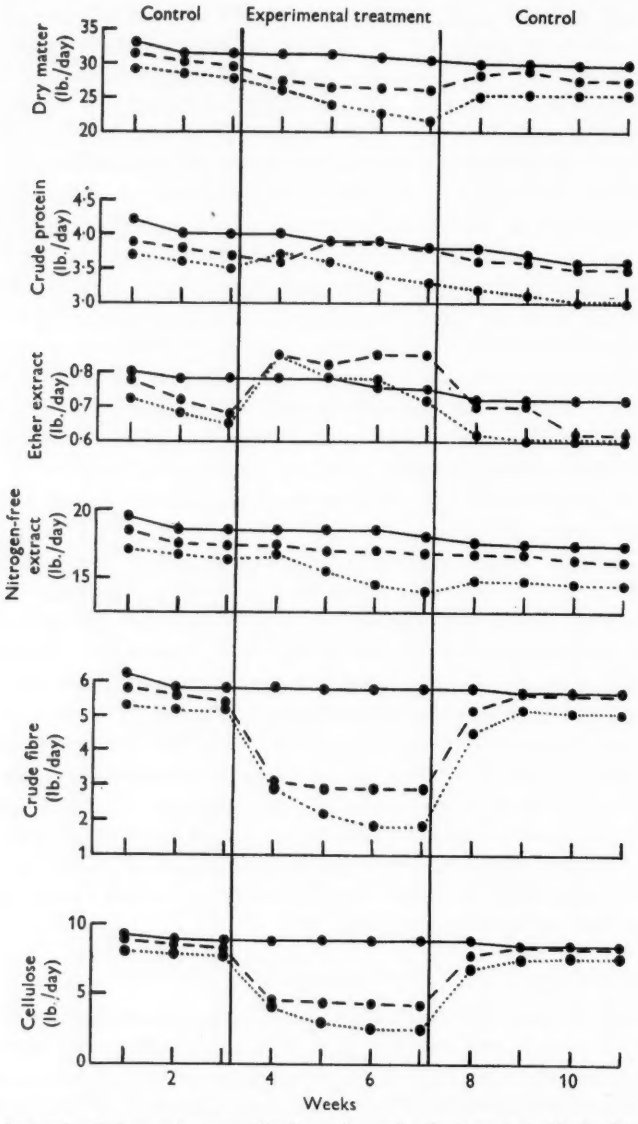

Fig. 3. The Reichert value, iodine value, saponification value and softening point of butterfat from groups of cows receiving daily diet A (about 18 lb. of hay), ●——●, diet B (6 lb. of hay), ●– – –●, and diet C (2 lb. of hay), ●····●, in the period of experimental treatment. During the control periods all the groups received 18 lb. of hay daily.

was fed there was no increased milk production to compensate for the drop in fat content, and therefore the fall in yield of fat was more marked than in group B.

Table 4. *The percentage fat content of milk from cows given diets low in hay and high in concentrates*

		Group A					Group B					Group C				
Week	Sample	Cow 1	Cow 2	Cow 3	Cow 4	Weekly mean	Cow 5	Cow 6	Cow 7	Cow 8	Weekly mean	Cow 9	Cow 10	Cow 11	Cow 12	Weekly mean
		Diet A (control)					Diet A (control)					Diet A (control)				
1	1	3·65	3·65	3·78	3·94	3·93	4·46	3·51	3·74	3·14	3·74	3·42	3·89	4·19	4·22	3·84
	2	3·98	5·38	4·12	3·49		4·10	4·41	3·78	3·34		3·89	3·48	3·91	3·80	
	3	3·78	3·92	4·12	3·38		3·68	4·28	3·40	3·05		3·54	3·62	3·98	4·14	
		Diet A (control)					Diet A (control)					Diet A (control)				
2	1	4·00	3·40	3·86	3·51	3·73	3·82	4·28	3·70	4·48	3·88	3·58	3·18	4·00	3·89	3·79
	2	3·70	3·02	4·70	3·30		3·40	3·71	3·70	3·98		3·72	3·65	4·70	3·79	
	3	3·82	3·24	4·38	3·78		3·50	4·75	3·45	3·75		3·65	3·22	4·18	3·91	
		Diet A (control)					Diet A (control)					Diet A (control)				
3	1	4·18	3·82	4·72	3·52	3·86	3·78	3·59	3·80	4·20	3·84	3·80	3·62	3·98	3·42	3·74
	2	4·20	3·49	4·35	3·20		3·72	3·96	3·70	4·80		4·02	3·42	4·05	4·02	
	3	3·88	3·25	4·24	3·52		3·86	3·74	3·58	3·38		3·84	3·36	3·74	3·65	
		Diet A (control)					Change of diet					Change of diet				
4	1	3·91	3·52	4·20	3·44	3·84	3·62	3·46	3·86	3·26	3·80	4·40	3·39	4·08	3·62	3·61
	2	3·95	4·28	4·19	3·30		3·72	3·22	3·78	7·22		3·66	2·82	4·40	4·14	
	3	4·04	3·52	4·36	3·32		3·34	4·38	3·62	2·19		3·02	—	3·32	3·22	
		Diet A (control)					Diet B (6 lb. hay)					Change of diet				
5	1	3·78	3·62	4·68	4·18	3·85	3·40	3·22	2·85	1·70	2·92	2·49	3·10	3·49	3·39	3·33
	2	3·76	3·20	4·12	3·28		3·38	3·38	2·28	2·31		2·34	3·32	3·50	3·14	
	3	3·72	3·90	4·42	3·48		3·51	2·99	2·18	3·82		4·18	3·40	3·86	3·75	
		Diet A (control)					Diet B (6 lb. hay)					Diet C (2 lb. hay)				
6	1	3·74	3·30	4·22	3·30	3·79	3·62	3·32	2·59	2·88	3·27	2·84	3·10	3·62	3·48	3·10
	2	3·72	3·50	4·46	3·38		3·34	3·11	2·85	3·30		2·92	3·10	2·96	3·16	
	3	4·00	3·88	4·26	3·70		3·38	3·79	3·52	3·51		2·55	3·15	3·46	2·85	
		Diet A (control)					Diet B (6 lb. hay)					Diet C (2 lb. hay)				
7	1	3·82	3·26	4·02	3·32	3·80	2·48	3·32	3·64	2·80	3·30	2·65	3·08	3·82	3·10	3·26
	2	3·68	3·65	3·89	3·60		3·28	3·52	3·08	3·45		2·79	3·28	3·58	3·89	
	3	4·16	3·89	4·66	3·60		3·40	3·48	3·72	3·48		2·70	3·30	3·70	3·25	
		Diet A (control)					Change of diet					Change of diet				
8	1	3·70	3·54	4·62	3·66	3·82	3·78	4·05	3·58	3·58	3·68	2·75	3·05	3·62	2·48	3·20
	2	3·72	3·84	4·50	3·50		3·38	4·08	3·38	3·48		2·88	3·32	3·81	2·75	
	3	3·92	3·16	3·94	3·75		3·50	4·10	3·30	3·96		3·06	3·30	3·79	3·58	
		Diet A (control)					Diet A (control)					Diet A (control)				
9	1	3·58	3·29	3·99	3·65	3·82	3·16	3·30	3·29	2·80	3·52	3·30	3·49	3·60	3·38	3·63
	2	3·88	3·72	4·94	3·72		3·82	4·26	3·20	4·54		3·82	3·50	4·02	3·80	
	3	3·89	3·12	4·45	3·58		3·68	3·02	3·40	3·78		3·78	3·44	3·46	3·99	
		Diet A (control)					Diet A (control)					Diet A (control)				
10	1	4·10	3·72	4·18	3·68	3·81	3·70	4·06	4·12	4·35	3·75	4·00	3·56	3·85	3·72	3·76
	2	3·60	3·50	4·60	3·85		3·44	3·75	3·11	3·59		4·08	3·39	3·90	3·70	
	3	3·55	3·60	4·28	3·08		3·79	4·24	3·75	3·10		3·92	3·40	3·74	3·86	
		Diet A (control)					Diet A (control)					Diet A (control)				
11	1	3·78	3·75	4·02	3·60	3·81	3·65	4·56	3·95	3·58	3·86	4·18	3·66	3·59	3·96	3·76
	2	3·68	3·65	3·98	3·50		3·74	3·71	3·88	3·82		3·42	3·58	3·70	4·09	
	3	4·04	3·72	4·38	3·62		3·80	3·88	3·82	3·96		3·74	3·56	3·82	3·84	

Table 5. *The percentage solids-not-fat content of milk from cows given diets low in hay and high in concentrates*

		Group A				
Week	Diet	Cow 1	Cow 2	Cow 3	Cow 4	Group mean
2	A (control)	8·88	8·72	9·45	9·09	9·04
3	A (control)	8·84	8·74	9·48	9·00	9·02
4	A (control)	8·91	8·82	9·48	8·94	9·04
5	A (control)	8·89	8·76	9·35	8·82	8·95
6	A (control)	8·96	8·66	9·58	9·00	9·05
7	A (control)	8·90	8·48	9·40	8·90	8·92
8	A (control)	8·86	8·76	9·48	8·91	9·00
9	A (control)	8·76	8·65	9·27	8·81	8·87
10	A (control)	8·41	8·48	9·57	8·90	8·84
11	A (control)	8·86	8·59	9·14	9·04	8·91
		Group B				
Week	Diet	Cow 5	Cow 6	Cow 7	Cow 8	Group mean
2	A (control)	8·81	8·96	8·84	9·05	8·92
3	A (control)	8·83	8·96	8·78	9·10	8·92
4	Change	8·92	8·95	8·84	9·36	9·02
5	B (6 lb. hay)	8·88	9·06	8·90	9·20	9·01
6	B (6 lb. hay)	8·80	8·72	8·97	9·10	8·90
7	B (6 lb. hay)	8·96	8·60	8·96	9·14	8·92
8	Change	8·83	8·87	8·89	9·30	8·97
9	A (control)	8·91	8·94	8·75	9·06	8·92
10	A (control)	8·78	8·88	8·67	9·01	8·84
11	A (control)	8·83	8·92	8·93	9·10	8·94
		Group C				
Week	Diet	Cow 9	Cow 10	Cow 11	Cow 12	Group mean
2	A (control)	9·00	8·42	9·25	9·20	8·97
3	A (control)	9·27	8·49	9·21	9·17	9·03
4	Change	9·26	8·36	9·26	9·11	9·00
5	Change	9·31	8·53	9·37	9·33	9·14
6	C (2 lb. hay)	9·11	8·44	9·08	8·97	8·90
7	C (2 lb. hay)	9·55	8·33	9·02	9·24	9·04
8	Change	9·37	8·24	8·81	9·19	8·90
9	A (control)	9·01	8·28	8·88	8·95	8·78
10	A (control)	8·96	8·66	8·67	9·34	8·91
11	A (control)	9·46	8·38	8·62	9·13	8·90

Decreases in the fat content of the milk and the yield of fat during this experiment were apparently rather smaller than decreases which have sometimes been observed previously on comparable diets (1, 2, 3 and possibly 5). Since few of these earlier experiments have been fully reported, it is impossible to compare results in detail. In the present instance all the cows received 30 lb. of mangolds and 4 lb. of a concentrate mixture balanced for milk production per 10 lb. of milk, but the hay, given to the control group A at the daily rate of about 18 lb. was reduced to 6 and 2 lb. for groups B and C respectively, and replaced by 9 lb. of a special concentrate mixture low in fibre and protein. The treatments were, therefore, less extreme than those used by others in the previous experiments. For example, the very marked decrease in milk fat percentage observed by Stoddard *et al.* (5) was the result of limiting cows to 6 lb. or less of hay daily, and allowing them to eat concentrates *ad lib.* and it is reported that some of the cows consumed as much as 45 lb. of concentrates daily. The present results appear to be more in keeping with the findings of

Loosli *et al.* (4), who obtained statistically significant differences between diets respectively high and low in hay but of approximately equal calorific value. No experiment has yet conclusively demonstrated that the decline in fat content is due to a low roughage intake *per se* rather than to an increased intake of concentrates, but the present experiment suggests that the decline can be produced without very large increases in dietary crude protein, although the amount of protein digested will have increased.

This experiment did not confirm the finding of Powell (1, 2) that the solids-not-fat content of the milk fell with diets low in roughage in the same way as did the milk fat content. This may have been due to the different length of the experiments; that of Powell lasted for whole lactations, whereas the present trial included only 18 to 21 days of full experimental feeding.

An examination of butter fat showed that when diets low in roughage and high in concentrates were given, there was a rise in the iodine value of the butter-fat. Stoddard *et al.* (5) found both a raised iodine value and a lowered Reichert value in butter-fat from cows on their experiment; however, in the present experiment a lowered Reichert value was only observed in the cows receiving 2 lb. hay daily. Since the proportion of unsaturated fatty acids in the butterfat apparently increased, as suggested by a lowered melting-point and the changes in Reichert value and iodine number discussed above, there are some grounds for suggesting that the decrease in the amount of milk fat was due to a decreased supply of some substance or substances used for the synthesis of the saturated acid portion of the fat. It has recently been shown that acetic acid is built up into milk fat by ruminants (Folley (13)), and Stoddard *et al.* (5) claimed that the depression of milk fat content due to diets low in hay and high in concentrates was corrected by the administration of acetic acid, but not of propionic acid, into the reticulo-rumen. It is not yet known whether the rate of production of acetic acid in the reticulo-rumen is changed by the diets under discussion.

SUMMARY

1. Twelve Shorthorn cows were used for an investigation into the effect of diets low in roughage and high in concentrates on the fat content of milk.

2. An experiment was conducted with initial and final control periods. During the control periods all the cows received daily 17 to 21 lb. of hay, 30 lb. of mangolds and 4 lb. of a balanced concentrate mixture per 10 lb. of milk. In the period of experimental treatment, lasting 4 weeks, a control group of four cows remained on this diet. Two other similar groups received daily only 6 and 2 lb. of hay, but this was supplemented with 9 lb. of a concentrate mixture low in fibre and protein. The same allowances of mangolds and of concentrates for production were given to these as to the control group.

3. The mean fat content of milk produced in the last 2 weeks of the period of experimental treatment by cows receiving the diets low in roughage was 0·49% (group receiving 6 lb. of hay) and 0·59% (group receiving 2 lb. of hay) below that produced by the cows receiving the control diet, these differences being statistically significant. In the group receiving daily 6 lb. of hay the drop in fat content was accompanied by an increase in the yield of milk and there was only a small fall in the yield of milk fat, but in the group receiving daily 2 lb. of hay there was a significant fall in the yield of fat. The latter fall represented a loss of 18·9% of the fat as compared with the control group. The fat content of the milk and the yield of milk rapidly returned to normal when the cows reverted to 18 lb. of hay per day.

4. The butterfat produced by the cows receiving diets low in roughage had a higher iodine value and lower softening point than that from the control cows. The fat from the group receiving daily 2 lb. of hay, but not that from the group receiving 6 lb. of hay, had a distinctly lower Reichert value than fat from the control group. The treatments caused no change in the saponification value.

5. The level of solids-not-fat in the milk was not affected by reduction in the roughage intake.

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461. 'BROKEN' OR 'BITTY' CREAM IN RAW AND PASTEURIZED MILK

By M. JEAN STONE and A. ROWLANDS
National Institute for Research in Dairying, University of Reading

INTRODUCTION

'Broken' or 'bitty' cream occurs in both raw and pasteurized milk. When the milk is shaken, the gravity cream breaks into particles of varying size which cannot be re-emulsified like the cream of normal milk. In hot tea or coffee, the particles float on the surface and are unsightly and objectionable. Milk in the initial stages of the fault is normal in flavour and titratable acidity; later a firm non-acid clot is produced. Not uncommonly, in bottled milk in which a compact cream layer has formed, the fault simulates that generally referred to as 'cream plug', though from the descriptions of this fault (1, 2, 3) it appears to be quite distinct from 'broken' or 'bitty' cream.

Casual inquiries have revealed that outbreaks of the fault are not infrequent throughout England and Wales, especially during spells of exceptionally hot weather, but little information is available in the literature regarding it. Its occurrence was first recorded in 1938 (4). According to this report, samples of milk exhibiting the fault were usually found to contain large numbers of aerobic spore-forming bacilli. In a further report 2 years later (5) it was stated that a specific organism, *Bacillus mycoides*, had been isolated from many of the samples. Davis (6) ascribed the fault to *B. mycoides*. He found that outbreaks of broken cream occurred only in hot weather, and that the initial colony count of milk which developed the fault was generally very low. He suggested that the spore-former, in the absence of other organisms, particularly acid producers, proliferated rapidly in milk above a critical temperature of 20° C. and caused sweet curdling within a very short time.

The work to be described was begun in the autumn of 1949. During the spring and early summer of that year spasmodic outbreaks of the fault had occurred in milk produced at the Institute farm. From routine weekly examinations of samples of the raw milk and the same milk after pasteurization, it was found that several of them, when stored at 22° C., had developed typical broken cream within 24 hr. of production or pasteurization. Spore-forming bacilli were frequently isolated from streak plates prepared at the end of this incubation period. The herd from which the milk was produced was machine milked, all the equipment being steam sterilized, and the milk pasteurized by the 'Holder' process and filled into cartons on the farm. The initial colony count of the raw milk samples rarely exceeded 5000 per ml. and that of the pasteurized samples was generally less than 150 per ml.

Later in the summer of 1949, when the weather was exceptionally warm, a local dairy distributing pasteurized milk experienced a serious outbreak of the fault. Samples of the bulk tanker milk received for processing were brought to the Institute for examination, together with samples of the bottled pasteurized milk. The former were heated to 80° C. for 10 min. and cooled at the processing depot before despatch. All these samples, when held at laboratory temperature (about 25° C.), developed broken cream within 24 hr. On

streak plates prepared at the end of this time aerobic spore-bearing rods were found to comprise the bulk of the bacterial flora. Representative colonies were isolated and kept for further study.

In addition, 12 cultures of aerobic spore-formers isolated from milk showing broken cream were kindly supplied by a large dairy in the Midlands, which also experienced an outbreak of broken cream during the same summer.

At the beginning of the present work, therefore, a total of 44 cultures of aerobic spore-forming bacilli, isolated from samples of raw and pasteurized milk in which the fault had developed, were available for study. Of this total, 17 were obtained from raw and pasteurized milk from the Institute farm, 15 from the local dairy and 12 from the dairy in the Midlands. The identity of these cultures was established and a study made of their ability in pure culture to produce broken cream in laboratory pasteurized, aseptically drawn milk.

Early in 1950, it was decided to extend the work to include the examination of milk from a group of farms supplying the local processing depot where the outbreak of broken cream had occurred the previous summer. Samples of raw milk from fifteen farms were examined twice during the period January to March 1950 and twice more between April and June. In this way it was hoped to obtain some indication of the distribution of organisms causing broken cream in milk from different farms both in winter and in summer. At the same time, the distribution of the organisms in bottled pasteurized milk was also studied.

EXPERIMENTAL

Purification of cultures

Single colonies of each type of organism present on the streak plates prepared from milk showing broken cream or sweet curdling were picked off into tubes of nutrient broth. After 24 hr. incubation at 30° C., one loopful of each culture was streaked again on 3% nutrient agar plates. If the culture then appeared pure, a single colony was transferred, through a second nutrient broth culture, to two 1·5% nutrient agar slopes; if not, the process was repeated until a pure culture was obtained.

Classification of cultures

The system evolved by Smith, Gordon & Clark (7) was adopted for the classification of our cultures. Their methods for identification of organisms of this group were also followed quite closely. The tests which we found most useful in our examination of cultures were:

Microscopic examination. Measurements of vegetative cells were made from growth on 1·5% nutrient agar slopes after incubation for 24 hr. at 30° C. To avoid shrinkage of the cells, a negative stain—nigrosin—was used and the film was air dried.

According to Gibson & Topping (8) and Smith *et al.* (7), the cells of *B. megatherium*, *B. cereus* and *B. mycoides* show a characteristic granular appearance of the protoplasm when lightly stained with fuchsin. Observations were made on growth from 48 hr. glucose nutrient agar slope cultures. Smears were dried and fixed in a flame and flooded with dilute fuchsin for 30 sec. At the same time, the shape and position of the spores as well as the shape of the sporangia were noted.

Macroscopic examination. The colonial appearance of the cultures on the surface of

3% nutrient agar was noted, but proved to be of little value in classification except in the case of *B. cereus* var. *mycoides* which produces characteristic rhizoid colonies. The nature of the growth on glucose nutrient agar and on glucose nitrate agar was also, in our experience, found to be of little value in the classification of cultures.

Physiological examination. Tests for the ability to grow at pH 6, and on glucose nitrate agar, to hydrolyse starch, to produce acetyl-methyl carbinol and to ferment sugars were carried out strictly according to the methods of Smith *et al.* Bearing in mind their observations, arabinose and xylose were the only sugars tested. For gelatin liquefaction the stab method was used in preference to the plate method. Cultures were also examined for haemolytic activity by streaking one loopful of a 24 hr. nutrient broth culture on the surface of 3% nutrient agar containing 5% ox blood. Wherever possible, 3% nutrient agar was used throughout this work to limit the spreading growth of the cultures (9, 10).

Lecithinase production was studied using the egg-yolk medium of McGaughey & Chu (11). Inoculated tubes were incubated at 22, 30 and 37° C., and cultures showing no reaction after 7 days were recorded as non-lecithinase producers.

The action of pure cultures of aerobic spore-bearers on milk

As we were interested mainly in the action of cultures on the surface cream layer, it was essential to use milk whose creaming ability was impaired as little as possible during sterilization. Samples of the bulk milk from a single cow in mid-lactation milked with a steam sterilized milking machine were transferred with a sterile dipper from the milking bucket to a narrow-necked bottle of approximately 200 ml. capacity. Within ½ hr. of sampling, the milk was preheated in a water-bath to 65° C., using a similar bottle filled with water as control. When the required temperature had been attained, the cotton-wool plug was replaced with a tightly fitting rubber stopper and the bottle immersed in a constant temperature water-bath at 62·5 ± 0·5° C. for 30 min. On removal from the water-bath, the bottle was cooled in running water, incubated overnight at 18° C. to induce germination of any spores, and then reheated at 62·5° C. for 30 min. After cooling, samples were stored in the ice-chest until required for use.

The sterilized milk was dispensed in 25 ml. quantities, using a sterile pipette, into 150 ml. conical flasks. Experience showed that this provided optimum conditions for the observation of the action of cultures on the cream layer.

In the early stages of the work 25 ml. of milk was inoculated with one loopful of an 18 hr. nutrient broth culture between 9 and 10 a.m., the samples were incubated at 22° C. and observed for the development of broken cream and sweet curdling after 6, 24, 30 and 48 hr. However, it was found that the more active strains of *B. cereus*, while producing no reaction in 6 hr., completely curdled the milk within 24 hr. Therefore, in later experiments the procedure was modified. A needle was used instead of a loop to inoculate the milk from a 6 hr. nutrient broth culture between 4 and 4.30 p.m. The inoculated milks were incubated at 22° C. and observed after 18, 24 and 42 hr. With this method it was possible to make observations for broken cream sufficiently early after inoculation even with the most active cultures.

The formation of broken cream was noted by gently swirling the contents of the flasks. Normally slight agitation suffices to mix the cream layer evenly in the body of the milk, but when broken cream has developed the cream layer breaks into particles of varying

size which cannot readily be mixed with the milk. The samples were discarded after 48 hr. incubation. Controls of uninoculated milk were used in every experiment and about half of the positive cultures, selected at random, were re-examined at least once.

The incidence of aerobic spore-formers in raw and pasteurized milk

Examination of raw farm milk supplies. All samples were taken from individual churns on arrival at the creamery. With very few exceptions morning milk was examined, so that samples were obtained within 4 or 5 hr. of production. A long-handled sterile dipper was used to mix the milk in the churn, then, without raising the dipper above the rim of the churn, a 25 ml. sample was transferred from it into a 150 ml. conical flask with a sterile pipette. Care was taken to prevent the milk touching the neck of the flask which, during subsequent heat treatment, would be above the level of the water. The flasks were then partly immersed in water at 70° C. for 10 min., a lead ring around the neck being used to weight the flasks. A similar flask containing 25 ml. of water and a thermometer served as control. Before heating, any milk on the neck of the flask was charred in a bunsen flame. The flasks were cooled in a shallow bath of cold water, then brought to the laboratory and held in the ice chest until 4.30 p.m. on the same day. They were then transferred to an air incubator maintained at 22° C. and examined after 18, 24 and 42 hr. for the presence of broken cream.

Isolation of causative organisms. After incubation for 42 hr., one loopful of the milk from each flask was transferred to 9 ml. of Ringer solution and then one loopful of the suspension was streaked on to egg-yolk agar (11). At the same time, a microscopic examination of a Gram-stained smear from each sample was made to obtain some indication of the type of organisms present.

The plates were examined after incubation for 24 hr. at 30° C. On this media, lecithinase producing cultures develop a characteristic cream-coloured opaque zone due to the aggregation of free fat released from the egg yolk by the hydrolysis of lecithin, while non-lecithinase producers exhibit no such zone formation. Representative colonies of each type were picked off into nutrient broth, further purified by streaking on 3% nutrient agar plates and finally stored on 1·5% nutrient slopes.

Classification of isolated cultures. On the basis of McGaughey & Chu's and our own observations that production of lecithinase is confined to the majority of strains of *B. cereus*, *B. anthracis* and *B. mycoides*, cultures showing this reaction at the time of isolation were not examined further. Lecithinase positive strains with rhizoid colonies were classified as *B. mycoides* and non-rhizoid strains as *B. cereus*. The non-lecithinase producers were examined by the methods detailed earlier.

Examination of bottled pasteurized milk. On each visit to the dairy for sampling producers' milk, samples of bottled T.T. pasteurized milk and ordinary pasteurized milk were also obtained for testing. The T.T. milk was the first to be processed each day and the non-designated was pasteurized immediately afterwards. The bottle taken for sampling was the second to be filled from each run.

The samples were well mixed by inverting the bottles several times, the caps carefully removed and 50 ml. of the milk transferred with a sterile pipette to a 150 ml. conical flask. The samples were subsequently treated exactly as described for the samples of raw milk.

Concentration of the lecithinase of Bacillus cereus

An attempt was made to obtain a concentrated preparation of the enzyme, lecithinase, produced by *B. cereus* and its variants, *B. anthracis* and *B. mycoides* (11). First, 1 l. of nutrient broth containing 0·2% dextrose was inoculated with 1 ml. of an 18 hr. broth culture of a particularly active strain of *B. cereus*, and incubated at 30° C. for 24 hr. It was then Seitz filtered to remove the cells. To obtain some indication of the amount of enzyme present, 1 ml. of the filtrate was added to 5 ml. of egg-yolk broth and the mixture held in a water-bath at 37° C. Where a faint but definite turbidity developed in the mixture after 1 hr. incubation, the lecithinase active filtrate was concentrated by the method of Chu (12). The concentrate was dialysed against glycerol at 0° C. and subsequently stored as a glycerol solution in the ice-chest. The final volume of concentrated solution obtained from 1 l. of broth filtrate was approximately 50 ml.

The action of lecithinase concentrate on milk

The action on milk of the active culture filtrate before concentration was first tested. Five ml. quantities of the filtrate were added to 25 ml. quantities of milk—sterilized by the method outlined earlier—in 150 ml. conical flasks. For each filtrate tested, six flasks were inoculated. Two, together with an uninoculated control, were incubated at 22° C., and the other two, also with a control, were incubated at 0° C. The flasks were observed daily for the development of broken cream.

The concentrated preparation was then tested for its action upon milk and upon cream. To 25 ml. of milk in a flask was added 5 ml. of the concentrated culture filtrate. This, together with an uninoculated control flask, was held at 22° C. and observed for the production of broken cream. To the cream obtained from centrifuging 100 ml. of milk and pipetting off the lower skim milk layer, was added 2 ml. of the concentrate and the cream was incubated, in the centrifuge tube, with a control tube at 37° C. Observations were made for the development of any change simulating broken cream.

RESULTS

The classification of the aerobic spore-bearers isolated from milk

During the present work a detailed study was made of 161 cultures of aerobic spore-forming bacilli isolated from raw and pasteurized milk. These included the 44 cultures which were available at the outset.

In Table 1 are given the more important tests used in our identification and the distribution of our cultures within the grouping proposed by Smith *et al.* Out of the total of 161 cultures, 96 were classified as *B. cereus*, 27 as *B. cereus* var. *mycoides*, 17 as *B. subtilis*, 11 as *B. sphericus*, 3 as *B. megatherium*, 2 as *B. pumilus* and 1 as *B. firmus;* the identity of the remaining 4 was not established.

It will be seen from the table that although 3 cultures did not produce a lecithinase reaction on egg-yolk media, they were classified as *B. cereus* because, according to the methods of Smith *et al.*, they were obviously members of this group.

The distribution of the different species of aerobic spore-formers in raw and pasteurized milk

A total of 60 samples of raw milk, taken on six different occasions between January and June 1950 from fifteen different farms, were examined for the presence of aerobic

Table 1

No. of cultures	Microscopic examination	Growth at pH 6	Growth on glucose nitrate agar	Starch hydrolysis	Gelatin hydrolysis	Xylose	Arabinose	V.P.	Haemolysis	Lecithimase activity	Identity of organism
93	3·75 μ–4·5 μ × 1·25 μ, protoplasm granular, spores oval, sporangia not swollen	+	–	+	+	–	–	+	+	+	*B. cereus*
3	3·75 μ–4·5 μ × 1·25 μ, protoplasm granular, spores oval, sporangia not swollen	+	–	+	+	–	–	+	+	–	*B. cereus* (atypical)
27	3·75 μ–4·5 μ × 1·25 μ, protoplasm granular, spores oval, sporangia not swollen, growth rhizoid	+	–	+	+	–	–	+	+	+	*B. cereus* var. *mycoides*
17	3·0 μ × 0·9 μ, spores oval, sporangia not swollen	+	+	+	–	+	+	+	–	–	*B. subtilis*
3	4 μ × 1·25 μ, protoplasm granular, spores oval, sporangia not swollen	+	+	+	+	+	+	–	–	–	*B. megatherium*
2	2·5 μ × 0·9 μ, spores oval, sporangia not swollen	+	–	–	+	+	+	+	–	–	*B. pumilus*
1	3 μ × 1 μ, spores oval, sporangia not swollen	–	–	+	+	–	–	–	–	–	*B. firmus*
11	3·75 μ × 1 μ, spores round, sporangia swollen	+	–	–	–	–	–	–	–	–	*B. sphaericus*

spore-bearing rods. As already described, the organisms were isolated from streak plates prepared after incubation of samples, heated initially to 70° C. for 10 min., at 22° C. for 42 hr. During the same period, 14 samples of bottled pasteurized milk were examined in a similar way. The 44 cultures isolated from milk in preliminary work were also taken into account and the distribution of the different types of aerobic spore-forming rods in raw and pasteurized milk is shown in Table 2.

Table 2

Source of culture	Total no. of cultures isolated	Number of					
		B. cereus	*B. mycoides*	*B. subtilis*	*B. sphaericus*	*B. megatherium*	Other species
Raw farm milk	100	51	23	10	9	1	6
Farm pasteurized milk	16	11	3	0	0	1	1
Bulk raw milk	8	8	0	0	0	0	0
Creamery bottled pasteurized milk	28	21	0	5	1	1	0
Creamery bottled pasteurized T.T. milk	9	5	1	2	1	0	0

The action of pure cultures of aerobic spore-formers on sterile milk

Preliminary observations were made using the 44 cultures originally available. At the same time, the reaction in milk of the following type cultures was investigated: *B. subtilis* N.C.T.C. no. 2586, *B. mycoides* N.C.T.C. no. 926, *B. megatherium* A.T.C.C. 1939, no. 943.

It was found that the 36 cultures classified as *B. cereus* and the 5 *B. mycoides* cultures, including N.C.T.C. no. 926, produced typical broken cream in sterile milk within 18 hr. at 22° C. followed by rapid sweet curdling, generally within 24 hr., at this temperature. The remaining cultures, 5 *B. subtilis* including N.C.T.C. no. 2586 and *B. megatherium* A.T.C.C. 1939, no. 943, produced no visible change in milk under the conditions of our experiments, within 48 hr. at 22° C.

Later, the action on milk of 52 of the cultures isolated from the 60 raw and 14 pasteurized milk samples was studied. Of these cultures, 19 were typical *B. cereus* or *B. mycoides* which produced broken cream in sterile milk within 18 hr. at 22° C. followed by rapid sweet curdling. Of the remaining cultures, which produced no reaction in milk within 48 hr., 3 were classified as atypical *B. cereus*, 12 as *B. subtilis*, 10 as *B. sphaericus*, 1 as *B. megatherium*, 2 as *B. pumilus*, 1 as *B. firmus* and 4 were not identified. The three atypical *B. cereus* cultures differed from the ordinary type only in their inability to produce a lecithinase reaction in egg-yolk broth. Repeated tests for the lecithinase activity at 22, 30 and 37° C. of these three cultures were consistently negative. It may be pointed out here that McGaughey & Chu (11) recorded the occurrence of occasional lecithinase negative strains of *B. cereus*. These three strains were the only non-lecithinase producers among the cultures of *B. cereus* isolated and studied in this work.

The incidence of broken cream in samples of raw and pasteurized milk

All but 13 of the samples of raw milk examined developed typical broken cream within 18 hr. when held at 22° C. The 47 positive samples were completely curdled within a further 24 hr. From streak plates prepared from these 47 samples, typical lecithinase

producing colonies of *B. cereus* and/or *B. mycoides* were isolated. These organisms were apparently present in pure culture in 39 of the 47 samples, but from each of the remaining samples, *B. sphaericus* was also isolated and, in addition, from one of these samples a culture of *B. subtilis* was obtained.

Only one of the 13 samples which did not produce broken cream proved to be sterile. *B. subtilis* was isolated from 7 of these samples, atypical *B. cereus* from 3 samples, *B. pumilus* from 2 samples, *B. firmus* from 1 sample and four organisms whose identity was not established were obtained from 4 samples.

With two exceptions, the 14 samples of pasteurized milk examined developed broken cream within 18 hr. at 22° C. followed by sweet curdling within 42 hr. Cultures of *B. cereus* or *B. mycoides* or both were isolated from streak plates prepared at the end of this incubation period from the 12 positive samples. Cultures of *B. subtilis*, *B. megatherium* and *B. sphaericus* were isolated from the 2 samples in which no broken cream developed. In all, 17 cultures of aerobic spore-formers were isolated from the 14 samples of pasteurized milk; 11 were identified as *B. cereus*, 1 as *B. mycoides*, 3 as *B. subtilis*, 1 as *B. sphaericus* and 1 as *B. megatherium*.

The number and percentage of samples developing broken cream, the source of the samples and the time of year at which the samples were taken is shown in Table 3.

Table 3

	Raw farm milk		T.T. pasteurized milk		Ordinary pasteurized milk	
	No.	%	No.	%	No.	%
	January to March 1950					
Samples which developed broken cream in 18 hr. at 22° C.	32	86·8	3	75	3	75
Samples from which *B. cereus* and/or *B. mycoides* isolated	32	86·8	3	75	3	75
Samples from which other aerobic spore-bearers isolated	12	31·57	2	25	1	25
	April to June 1950					
Samples showing broken cream in 18 hr. at 22° C.	15	68·15	3	100	3	100
Samples from which *B. cereus* and/or *B. mycoides* isolated	15	68·15	3	100	3	100
Samples from which other aerobic spore-bearers isolated	8	36·36	0	0	0	0

DISCUSSION

The results presented in this paper show that bacterial spores are commonly present in both raw and pasteurized milk. Indeed, our limited observations suggest that it is rather exceptional to find freshly produced raw or pasteurized milk devoid of spores. Thus 75% of the 25 ml. samples of raw milk and all of the 50 ml. samples of pasteurized milk examined were found to contain spores. Various workers have shown (13, 14, 15) that the number of spores present in fresh milk is very small, and therefore we considered it impracticable to attempt to estimate either the number of spores present initially in the samples, or the proportion of spore-forming organisms to the total bacterial flora.

Strains of *B. cereus* or *B. mycoides* constituted the majority of the cultures isolated. Thus, of the 161 cultures isolated (see Table 2), 123 were identifiable with one or other of these organisms. In this connexion, it is well to draw attention to the possible enrichment

effect of the methods used. Our results are based on isolations made after incubation of the samples at 22° C. for 42 hr., and it may be that this temperature favours the development of *B. cereus* and *B. mycoides* at the expense of the other types. Indeed, our results cannot in any way be interpreted in terms of the proportion of spores of the different types present in raw milk at the time of its receipt at the creamery or in the pasteurized milk at the time of bottling. It is evident, however, that aerobic spore-formers are commonly present during both winter and summer in samples of raw and pasteurized milk.

In particular, our results indicate that aerobic spore-formers other than *B. cereus* or *B. mycoides* are unlikely to play a dominant part in the spoilage of milk. With three exceptions, the cultures of *B. cereus* and *B. mycoides* examined had a pronounced effect upon milk; they produced the typical broken cream condition in sterile milk within 18 hr. at 22° C., followed a few hours later by sweet curdling, while, on the other hand, none of the 32 cultures of *B. subtilis*, *B. megatherium*, *B. pumilus*, *B. firmus* and *B. sphaericus* tested produced any physical change in sterile milk during incubation at 22° C. for a period of 48 hr. No observations were made as to whether or not these cultures had any effect upon the flavour of milk.

The evidence is fairly conclusive that *B. cereus* and *B. mycoides* are the only spore-forming bacilli capable of producing broken cream in milk and, moreover, that these two organisms exclusively are likely also to be the cause of sweet curdling in raw and pasteurized milk. Davis (6) ascribed this type of spoilage to *B. mycoides*, but our observations suggest that *B. cereus* is equally, if not more frequently, involved.

It is of interest that the three strains of *B. cereus* which did not produce a characteristic reaction in egg-yolk broth, also failed to produce typical broken cream in milk. This, coupled with the fact that all lecithinase positive strains of *B. cereus* and *B. mycoides* do produce broken cream, strongly suggests that the appearance of the fault is a direct result of the action of lecithinase on some milk constituent. Since most of the lecithin in milk, together with associated protein, constitutes the so-called surface or protective 'membrane' (16) around the fat globules, it may be that by the enzymatic hydrolysis of the lecithin the surface membrane is wholly or partially disrupted and an aggregation of the fat globules occurs. However, if this were the case, one would expect an 'oiling off' of the cream to occur when warmed above the melting-point. As this is not the characteristic appearance of broken cream in tea or coffee, it seems more likely that the fault results partly from the action of the lecithinase on the lecithin of the fat globule surface 'membrane' and partly from a coagulation of the casein associated with the 'membrane'. Mention has already been made of the rapid curdling of milk by all lecithinase producing strains of *B. cereus* and *B. mycoides*.

All attempts to produce broken cream either in milk or in centrifuged cream by the addition of a lecithinase concentrate were, however, unsuccessful. In all cases, the milk curdled within 48 hr., indicating that a strongly active proteinase was also present in the concentrated filtrate. A possible explanation of our inability to reproduce the fault may be that dispersal of the enzyme throughout the bulk of the milk, or even its adsorption on to particles of protein, leaves insufficient available to attack the lecithin effectively at the seat of the reaction—the fat globule surface. When, however, the fault occurs naturally, the lecithinase producing bacteria are carried to the surface with the rising fat globules and, because of their strongly aerobic nature, it is probable that their enzyme production in the surface layers is rapid, a condition which cannot readily be reproduced artificially.

Our evidence in support of the part played by lecithinase in the production of broken cream, though indirect, is substantiated by the fact that the condition develops only in the presence of lecithinase producing strains of *B. cereus* or *B. mycoides*, and that the three lecithinase negative strains of *B. cereus*, as well as all other non-lecithinase producing species of the aerobic spore-forming bacilli, are unable to produce broken cream.

In view of the prevalence of broken cream, its prevention or suppression is of major importance to the milk distributor. From our limited observations of the widespread occurrence of the causative organisms in both raw and pasteurized milk, and bearing in mind that these organisms occur in milk mainly in the form of spores, prevention of infection of milk seems to present an insurmountable problem. Our attention has therefore been directed to the suppression, or at least to the possibility of delaying the onset of the fault sufficiently long to allow the milk to be consumed without the fault becoming objectionable. So far, we have been able to make only a few observations along these lines. In practice, broken cream mainly occurs in unusually hot weather, and this suggests that the fault only attains practical significance when the temperature of storage of the milk permits rapid multiplication of the surviving bacteria.

Pasteurized milk is usually bottled and held until the following morning before distribution to the consumer. Control of temperature, once the milk has been delivered, is difficult if not impossible, for the majority of householders have no cold storage facilities. Control of temperature prior to distribution might, however, play an important part in delaying the development of the fault. With this object in mind, we have made a few preliminary investigations.

Using a pure culture of *B. cereus* isolated from raw farm milk, approximately 10,000 spores per ml. were added to 200 ml. of sterile whole milk. The suspension was then pasteurized at 62·5° C. for 30 min., after which it was cooled in iced water for 15 min. The milk was then divided into three tubes, one of which was held in a water-bath at 5° C., one at 10° C. and one at 15° C. After 24 hr. storage at these temperatures, each portion was again divided into three tubes. These were incubated at 15, 18 and 22° C. for a further 24 hr., after which time observations were made for broken cream and sweet curdling. The total number of organisms and also the number of spores were estimated by poured plate counts immediately after pasteurization and then after 15 min., 6, 24, 30 and 48 hr. The spore count was obtained by heating 5 ml. quantities of milk in a test-tube to 80° C. for 10 min. before plating.

It will be seen that a total of nine different temperature combinations was used in order to simulate some of the conditions of storage under which bottled pasteurized milk might be kept prior to distribution and during the first 24 hr. in the hands of the consumer. The results are summarized in Table 4. It would seem that efficient refrigeration of pasteurized milk pending delivery has an important bearing on the occurrence of broken cream. Our observations indicate that, following preliminary storage of milk at 5° C. or lower, the fault is unlikely to develop within 24 hr. when the milk is kept by the consumer at a temperature as high as 18° C. However, a preliminary storage period at 15° C. followed by storage at 15, 18 or 22° C. for 24 hr. does not prevent the development of broken cream.

Provided milk is adequately refrigerated before distribution, it seems likely that in most households it may be stored at a temperature low enough to prevent the onset of the fault except in hot weather when, without artificial refrigeration, the prevention of broken cream may be virtually impossible.

Table 4

No. of samples examined	Initial inoculum of spores per ml.	Temperature of storage (° C.) 0–24 hr.	24–48 hr.	Visual change in milk after 48 hr.
4	10,000	5	15	No broken cream
			18	No broken cream
			22	Broken cream
		10	15	No broken cream
			18	Broken cream
			22	Broken cream
		15	15	Broken cream
			18	Broken cream
			22	Pronounced broken cream (Milk almost clotted)

SUMMARY AND CONCLUSIONS

Of 161 cultures of aerobic sporeformers isolated from raw and pasteurized milk 96 were found to be *B. cereus*, 27 *B. mycoides*, 17 *B. subtilis*, 11 *B. sphaericus*, 3 *B. megatherium*, 2 *B. pumilus*, 1 *B. firmus* and 4 were unidentified strains.

Typical broken cream followed by rapid sweet curdling of sterile milk was produced by 36 of 39 strains of *B. cereus* and 5 strains of *B. mycoides* examined. Three strains of *B. cereus* failed to produce broken cream. These 3 strains were atypical in that they failed to produce lecithinase, unlike the remaining 93 strains isolated during this study. Other species of aerobic sporing bacilli tested, including 17 strains of *B. subtilis*, 10 of *B. sphaericus*, 2 of *B. megatherium*, 2 of *B. pumilus* and 1 of *B. firmus*, all failed to produce broken cream and in fact produced no visual change in milk within 48 hr. at 22° C.

Under the conditions of our experiments, 48 out of 60 samples of fresh farm milk and 12 out of 14 samples of freshly bottled pasteurized milk developed broken cream within 18 hr. at 22° C. followed by sweet curdling within a further 24 hr. Strains of *B. cereus* or *B. mycoides* were isolated from all samples showing broken cream, but only 3 atypical strains of *B. cereus* were isolated from the 14 samples which did not develop the fault.

It seems that the production of broken cream is in some way associated with the production of lecithinase by *B. cereus* and *B. mycoides*. This view is supported by the fact that three non-lecithinase producing strains of *B. cereus* were also unable to produce broken cream. However, attempts to reproduce the fault by adding a concentrated culture filtrate to sterile milk or cream were unsuccessful.

Because of the widespread distribution of spores of *B. cereus* and *B. mycoides* in both raw and pasteurized milk, the absolute prevention of broken cream does not seem possible, but preliminary observations suggest that maintenance of milk at 5° C. or lower from the time of pasteurization until delivery to the consumer may sufficiently delay the onset of the fault to obviate the difficulty in practice except in very hot weather in households where insufficient care is taken to keep milk cool.

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462. THE KEEPING QUALITY OF COMPOSITE MILK

By J. BABAD and A. SHENHAV-HETMAN
Dairy Research Laboratory, Agricultural Research Station, Rehovoth, Israel

(With 5 Figures)

INTRODUCTION

A serious deficiency in milk supply has been prevalent in Israel for the last two years because of the pronounced rate of increase of the population. The Ministry of Rationing and Supply asked us therefore to investigate the possibility of applying milk-powder for drinking purposes, because the increase in domestic herds, combined with the import of cattle, is not enough to keep up with the rate of population increase.

The use of milk-powder is increasing in the whole world. From 1937 up to 1947 the use of milk-powder for human consumption has increased almost threefold. More than half of this production is skim milk-powder (1). Until a few years ago its most important use was in the bakery and in ice-cream manufacture. In more recent times its use has increased very rapidly in cheese-making (cottage cheese) and various types of cultured butter milk even in the U.S.A., a country rich in milk. The latter use is specially recommended by Costilow *et al.* (2), who claim that it is better to use good milk powder whose standard is determined and unchanging than fresh milk whose quality changes and is not guaranteed.

It is known that the nutritive value of the milk decreases almost insignificantly when it is converted to powder. The average composition of skim milk is given in Table 1 (4).

Table 1. *The average composition of skim milk powder*

Constituent		Quantity in 100 g. powder
Water (g.)		3·0
Protein (g.)		36·9
Lactose (g.)		51·0
Fat (g.)		0·9
Salt (g.)		8·2
Calcium (g.)	1·3	
Phosphorus (g.)	1·0	
Vitamin A (i.u.)		40·0
Riboflavin (mg.)		2·0
Thiamine (mg.)		0·35
Niacin (mg.)		0·85
Calories		350·0

This table indicates that skim milk-powder is a source of proteins, sugar, calcium and vitamins of the B complex. Milk proteins especially have a high nutritive value and supplement the proteins of lower value (i.e. of the cereals and vegetables).

The aim of the experiments outlined below was the investigation of some of its properties, and especially the keeping quality of composite milk (fresh milk mixed with varying quantities of skim milk-powder), as compared to natural milk.

At the end of 1949 we began our attempts to introduce into the dairies composite fluid

milk made up of fresh milk and skim milk-powder, which will be called henceforth 'composite milk'.

In the course of our work we learned that recently reconstituted whole milk and skim milk is being used in several countries such as Mexico (1), Alaska, the South-Pacific, Japan and occupied Germany (5). Details on the preparation of the milk were not given, but the emphasis was on the use of high-quality milk-powders. The above is generally impossible under our conditions because the milk-powders received in this country are mainly not of high quality for reasons beyond the scope of this paper.

We investigated three mixtures:

(*a*) Special concentrated milk: fresh milk with 3% fat to which skim milk-powder was added to a 24% solids content, because this concentration was originally intended for delivery to the housewife who would dilute it at home. This plan was abandoned for practical reasons.

(*b*) Special diluted milk: special concentrated milk diluted to 12% solids and 1·5% fat.

(*c*) Composite milk which contains only 0·75% fat but the solids content is adjusted to 13·5%.

Finally we conducted a series of experiments on mixtures of fresh milk with skim milk-powder and delactosed milk-powder, and with the latter we were able to increase the solids up to 17% without making the milk too sweet. The partially delactosed milk-powder used in these experiments was obtained from the Milk Research Corporation, Petaluma, California. This powder has a lactose content of about 32% as compared to 51% in normal skim milk-powder.

The composition of the mixtures except the one with the delactosed skim milk-powder was adjusted so that their calorific value approximated to that of fluid whole milk consumed in Israel. Table 2 shows the calculated calorific values of the various mixtures; that of reconstituted milk is given for comparison.

Table 2. *Mean calorific value of the various mixtures (calculated)*

Kind	Skim milk powder (kg.)	Fresh milk of 3% fat (l.)	Content (%) Solids	Fat	Protein	Lactose	Ash	Calories per l.
Fresh milk	—	100	11·55	3·0	3·0	4·8	0·75	58·00
Special diluted milk	8	50	13·5	1·5	4·45	6·48	1·04	57·8
Composite milk	11·4	25	13·8	0·75	5·20	6·95	0·90	55·5
Reconstituted milk	13·9	—	13·5	0·15	5·15	7·15	1·15	50·5
Delactosed C.M.	17·0*	25	17·2	0·80	8·00	7·40	1·00	69·2

* The skim milk powder used for the delactosed composite milk contained 10·0 kg. normal skim milk powder and 7·0 kg. partially delactosed skim milk powder.

EXPERIMENTAL

Materials

(*a*) The fresh milk was obtained from the 'Tnuvah' Dairy at Rehovoth, its fat content was about 3%.

(*b*) The skim milk powders were products of 'Cow and Gate', 'Land-O-Lakes' and 'Minnesota Valley' factories, imported from the U.S.A. Their moisture content was 3–5%.

(*c*) The 'special concentrated milk' (S.C.M.) was prepared by adding 14 g. milk-powder to 100 ml. of fresh milk and dissolving the powder in a Waring blender. This milk

contained about 24% solids and had the normal fat content of milk which was about 2·8 %.

(*d*) 'Special Diluted Milk' (S.D.M.) was prepared by diluting the S.C.M. with an equal volume of water. This gave a solids content equivalent to that of fresh milk—12 % solids and 1·4% fat.

(*e*) 'Composite Milk' (C.M.) was prepared by adding 45 g. milk powder to 100 ml. fresh milk and diluting with water to 400 ml. (Solids content was 13·5 %, and fat content, 0·75 %.)

(*f*) The water used throughout the experiments was freshly boiled tap water, cooled before use.

Methods

The samples were pasteurized at 63° C. for 30 min. in 750 ml. Erlenmeyer flasks held in a water-bath. Each flask contained 500 ml. of the appropriate milk. The flasks were plugged with cotton-wool and stored at summer room temperature (21–23° C.), winter room temperature (17–18° C.) and cellar temperature (10–12° C.). In each series four samples of unpasteurized and four of pasteurized milk were investigated. These were:

(1) Raw and pasteurized fresh milk.
(2) S.C.M., pasteurized and unpasteurized.
(3) S.D.M., both pasteurized and unpasteurized.
(4) C.M. pasteurized and unpasteurized.

The following determinations were made just after the samples were prepared and at intervals until the milk curdled:

Titratable acidity (SH°) was determined by titrating 50 ml. of milk with N/4-NaOH, the indicator being 0·5 ml. of 1% alcoholic solution of phenolphthalein. The results are expressed in SH°, which is the quantity of N/4-NaOH required for titrating 100 ml. milk under the same conditions (6).

Composite milk samples which contained a double solids content of natural milk were diluted with an equivalent volume of water before the SH determination. It must therefore be emphasized that wherever reference is made in the present paper to SH° of S.C.M., we mean SH° for the diluted sample.

pH determinations were carried out with the Beckman Model G pH meter, with a glass electrode.

Lactic acid was determined by the method of Heinemann (7), which is based on the formation of a coloured complex between veratrol and acetaldehyde obtained by the oxidation of the lactic acid. The intensity of the red colour formed was measured with the Lumetron type photoelectric colorimeter, the filter being 515 mμ. The calibration curve was made with calcium lactate.

Alcohol precipitation test (A.P.T.) was determined by the addition of 1 ml. milk to 1 ml. of 50, 68, 75, 85, 95 % alcohol respectively, and examining the solution for precipitated particles on the walls of the test-tubes.

Clot on boiling (C.O.B.) was determined by holding an ordinary test-tube with 5 ml. of milk in a boiling water-bath for 5 min.

In the last two determinations (A.P.T. and C.O.B.) a positive sign (+) indicates curdling and a negative sign (−) indicates that the milk had not yet clotted.

Reductase test was done in duplicate according to the method mentioned by House (8).

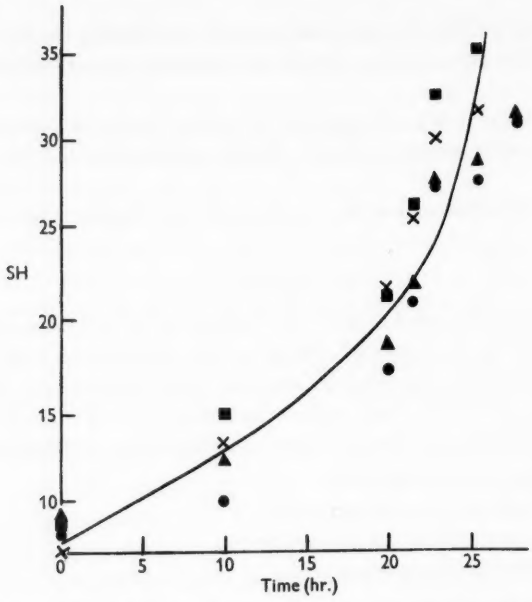

Fig. 1. Variation of SH with time of various milk mixtures stored at 23° C. ×, fresh milk; ■, special diluted milk; ●, composite milk; ▲, special concentrated milk.

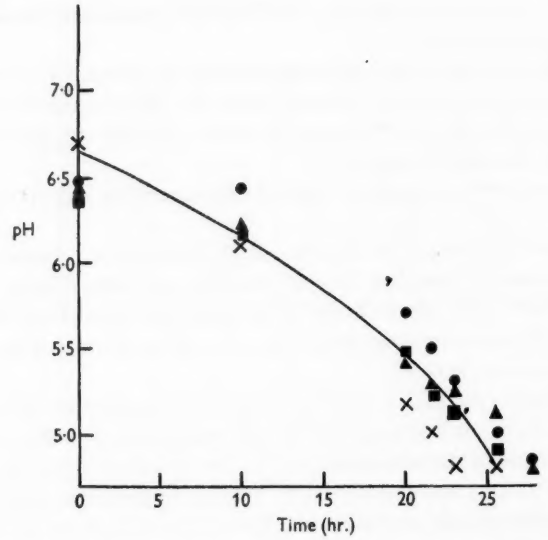

Fig. 2. Variation of pH with time of various milk mixtures stored at 23° C. ×, fresh milk; ■, special diluted milk; ●, composite milk; ▲, special concentrated milk.

Forty ml. of milk in a sterile test-tube were mixed with 1 ml. of 0·015 % aqueous methylene blue. The test-tubes were placed in a water-bath at 38–40° C. and the changes in the blue colour until its complete reduction were noted.

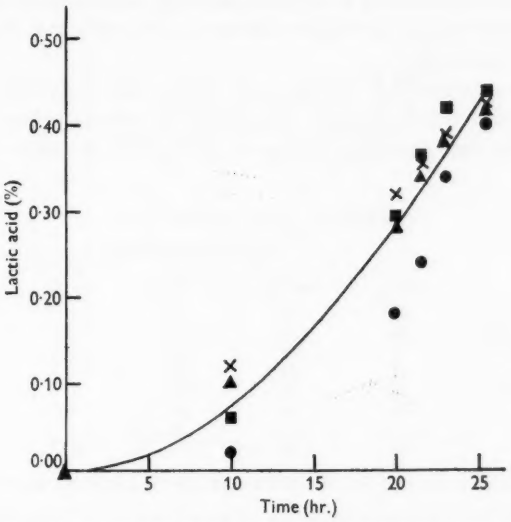

Fig. 3. Variation of lactic acid content with time of various milk mixtures stored at 23° C. ×, fresh milk; ■, special diluted milk; ●, composite milk; ▲, special concentrated milk.

RESULTS AND DISCUSSION

The results of SH, pH and lactic acid determination for the unpasteurized samples kept at 23° C. are given in Figs. 1–3, respectively. These are the averages of five series of experiments. The final point in all figures indicates curdling. The results for the other storage temperatures were of the same character, but the changes were proportionally slower. The pasteurized milk showed no change with time.

Although there are some differences between the various samples, they should not be considered as significant. The general trend of the changes seems to be the same in all cases. There was some indication that the keeping quality of the unpasteurized C.M. was the best, the reasons for which were not clear.

Practical considerations led us to assume that the milk would not reach the consumer later than 40 hr. after preparation; our experiments therefore were carried out mainly during this period. Usually the pasteurized milk was almost unchanged after 48 hr. at room temperature (23° C.). The differences between the series were usually small and of the same order of magnitude as the differences between the various kinds of milk.

Clot on boiling tests of all samples of pasteurized milk were negative for the duration of the experiment which was 40 hr. at 23 and 18° C. and 60 hr. at 10° C.

The times of storage until positive C.O.B. for the unpasteurized samples at 23° C. were as follows: raw milk, 21 hr.; S.C.M., 23 hr.; S.D.M., 25 hr.; C.M., 25 hr.

C.O.B. time was considered as the end-point of keeping quality because it was assumed that if the milk were distributed unpasteurized, it would be cooked before consumption.

The titratable acidity and C.O.B. tests were highly correlated. It could be concluded that 19–21 SH° was the transition point, after which the milk would clot on boiling.

No correlation was found between C.O.B. results and the A.P.T. (with the various alcohol concentrations). The A.P.T. became positive 5–10 hr. before C.O.B.

In view of the results presented in a recent paper by Rowlands, Barkworth, Hosking & Kempthorne (9) about the high correlation between A.P.T. and C.O.B., we intend to investigate this problem separately.

The S.C.M. showed appreciably shorter A.P.T. times. This is evident from the fact that its protein concentration is high, hence its stability to alcohol is lower.

Table 3 shows the time (hours) of storage at 23° C. until a positive A.P.T. reaction was observed.

Table 3. A.P.T. *versus* C.O.B.

Kind of milk	Alcohol concentration					C.O.B.
	50 %	68 %	75 %	85 %	96 %	
Raw milk	12	8	5	0	0	21
Fresh pasteurized milk	38	36	21	0	0	–*
S.C.M. unpasteurized	4	2	0	0	0	23
S.C.M. pasteurized	21	21	10	0	0	–*
S.D.M. unpasteurized	20	19	10	6	2	25
S.D.M. pasteurized	36	36	23	8	2	–*
C.M. unpasteurized	20	19	12	6	2	25
C.M. pasteurized	40	38	27	10	2	–*

* C.O.B. negative during the duration of experiments which was 40 hr.

The reductase test for raw milk as brought to the laboratory gave results indicating considerable bacterial contamination. The methylene blue was reduced after 1–3 hr. (at 37° C.). The S.C.M., apparently due to its raw milk content, gave results only slightly better than those for raw milk. Reduction time was 3–4 hr. for S.C.M.

Only S.D.M. and C.M. gave methylene-blue reduction times given by good milk according to definition, i.e. 5½ hr. Probably this was the effect of dilution.

Reductase tests carried out after 24 hr. storage at 23° C. on the unpasteurized milk samples gave the following results:

Raw milk	¼–½ hr.
S.C.M.	¾–1¼ hr.
S.D.M. and C.M.	1½–2 hr.

All the pasteurized samples gave methylene-blue reduction times of more than 8 hr. at the end of their storage (40 and 60 hr.) at the various temperatures.

Finally it may be stated that the keeping quality, as measured by C.O.B., of the unpasteurized S.D.M. and C.M. was the more satisfactory in comparison with the other unpasteurized samples. Raw milk and unpasteurized S.C.M. had approximately the same quality.

The pasteurized samples kept well in all cases (effectiveness of pasteurization).

While the main purpose of the experiments described was to study the keeping quality of the various milk mixtures, some subsidiary conclusions may be drawn from consideration of the results.

Figs. 4 and 5, in which the relation between percentage lactic acid and pH and SH respectively has been plotted, show that there is quite a definite correlation between these

values. There is, however, one strange phenomenon: the similar behaviour between S.C.M. and other samples as given in Fig. 4.

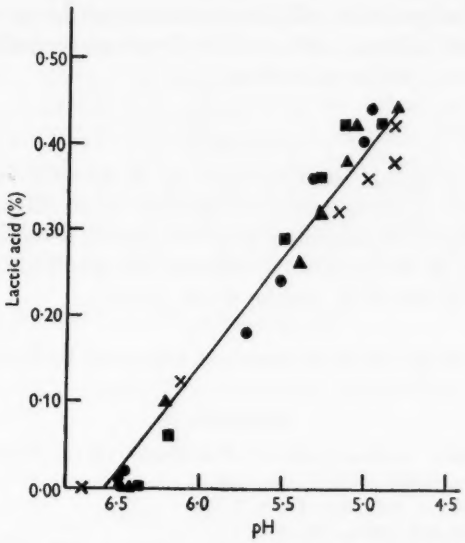

Fig. 4. Correlation between lactic acid concentration and pH of various milk mixtures stored at 23° C. ×, fresh milk; ■, special diluted milk; ●, composite milk; ▲, special concentrated milk.

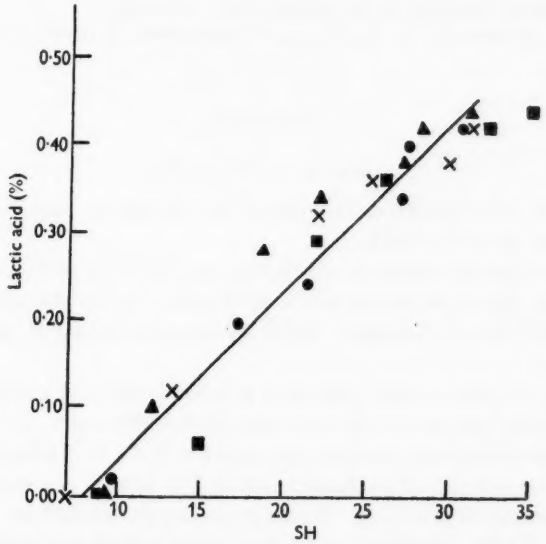

Fig. 5. Correlation between lactic acid concentration and SH of various milk mixtures stored at 23° C. ×, fresh milk; ■, special diluted milk; ●, composite milk; ▲, special concentrated milk.

This finding does not conform with the result of potentiometric titration of S.C.M. with lactic acid in which almost double quantities were needed as compared with the titration

of S.D.M. Some explanation might perhaps be found by the assumption that, while in the natural souring of milk there should be an equilibrium at each moment, this is not the case in the potentiometric titration. It is known that in the latter case no equilibrium is reached in the colloidal system of milk for the duration of the experiment. A potentiometric titration of milk allowing sufficient time for complete equilibrium to be obtained is not possible because of the necessary delay.

SUMMARY

The keeping quality of various mixtures, made up of raw milk and skim milk-powder in varying proportions, has been studied. It has been found that the keeping quality of unpasteurized samples containing milk powder was slightly better than that of raw milk. The keeping quality of the pasteurized mixtures was satisfactory during the time of storage for 40 hr. at 23 and 18° C. and for 60 hr. at 10° C.

We wish to acknowledge the suggestions and help given by Mr N. Sharon-Shtrikman.

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APPENDIX

LARGE-SCALE PRODUCTION

As a result of the investigation carried out at the laboratory, large-scale production of C.M. was begun at the end of 1950.

At the present time the contents of this C.M. per 100 l. are as follows: 25 l. raw milk (3 % fat); 11·4 kg. skim milk-powder and water to make up the final volume. The calorific value of this milk is 560 ± 2 Calories/l., which is equivalent to that of local fluid milk with 3 % fat.

In dairies, the composite milk is prepared as follows: the milk powder is added to the natural milk and the product is well mixed and diluted with water. It is then pasteurized by the holding or the H.T.S.T. methods and cooled to 4° C. It is filled into bottles of ¼ l. capacity which are sealed with cardboard covers. The bottles are washed and sterilized in a chlorine solution before filling. The milk reaches the market as 'special composite milk' which has the fat and solids non-fat percentage printed on the label. Another way of distribution of the C.M. is in 30 l. cans for schools and immigrant camps.

Generally, low-heated milk-powder is used, even though a good product can be obtained with the high-heated powders when they are in good condition and fresh. Whole milk-powder could not be used mainly because off flavours are always noted in this

powder when it is not of the best quality; this latter is not available to us in large quantities. The C.M. has been well accepted by the population. It is tasty, although there have been some complaints that it is too sweet. It is hoped that this fault will be eliminated when we can obtain sufficient quantities of delactosed skim milk-powder. This powder will be used for the production of another kind of C.M. which is less sweet and has a higher nutritional value, with 17·0 % total solids and 0·75 % fat.

There is a constant bacteriological control of the C.M. distributed. Phosphatase tests were always negative signifying satisfactory pasteurization. Coliform organisms were generally not found in a dilution of 1 : 1000, but found in dilution 1 : 100, signifying that the milk was contaminated after pasteurization, but not dangerously.

At the beginning of production approximately 25,000–30,000 l. of C.M. were produced daily. This is a considerable quantity as the total daily consumption of milk in Israel is less than 200,000 l.

The broadening of the above project is being planned.

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637.128.9:532.13

463. CHANGES WITH TEMPERATURE IN THE VISCOSITY OF WHOLE MILK

By C. P. COX
National Institute for Research in Dairying, University of Reading

(With 2 Figures)

1. INTRODUCTION

It is well known that the viscosity of milk changes with a number of factors, important ones being temperature and composition. Practical interest has been largely concerned with the viscosities of cream, evaporated milk and skim milk. Investigations on the changes with temperature in the viscosity of whole milk may, however, have applications, e.g. in control mechanisms for flow pasteurization processes, and some experiments on the viscosity of whole milk have been reported (Soxhlet (1), Kobler (2), Taylor (3), Tapernoux & Vuillaume (4), Spöttel & Gneist (5)). In addition, a careful experiment on the viscosity of homogenized milk was carried out by Caffyn (6). The experimental results given by Soxhlet (1) and Taylor (3) have been extensively quoted (Associates of Rogers (7), Davies (8), Petersen (9), Castle & Glücksmann (10), Harper (11), Davis (12)), and because of some inconsistencies the data available on the viscosity-temperature relations of whole cows' milk have here been re-examined.

2. RE-EXAMINATION OF THE DATA*

Soxhlet (1)

Measurements were made of the times of flow of 75 c.c. each of water and milk, using the Reischauer capillary viscometer, thermostatically controlled. The capillary was mounted vertically because only relative values were being investigated. Duplicate times of flow were obtained at 5° C. intervals for temperatures from 0 to 30° C., and at 25° C. a triplicate determination was made of the time of flow for water. The maximum difference between replicate readings was 0·53 %, at 20° C. The ratio of the time of flow for milk to that for water at the corresponding temperature was then calculated and given as the relative viscosity of the milk. Soxhlet's figures for this ratio are shown in Table 1 and in Fig. 1 in the Appendix. At 20° C. the times of flow were 375 and 794 sec. for water

Table 1. *Ratio of the time of flow of milk to the time of flow of water at different temperatures (Soxhlet)*

Temperature (° C.)	0	5	10	15	20	25	30
Relative time of flow	2·211	2·077	1·906	1·887	2·117	1·759	1·690

and milk respectively, and the corresponding relative time of flow, 2·117, is clearly anomalous, being higher than that for all preceding temperatures except 0° C. For further examination, curves were plotted showing time of flow against temperature. Within experimental error, the points for the milk could be fitted by a smooth curve showing a decrease in time of flow with increase in temperature. A corresponding smooth curve

* Attention has been chiefly confined to experimental methods and numerical results.

for water could not be obtained without omitting the time of flow for water at 20° C. given in Soxhlet's paper.

If η is the viscosity, ρ the density and t the time of flow, these quantities are connected under the circumstances of such experiments, neglecting small corrections, by a relation of the form

$$\eta = k_1 \rho t, \quad (1)$$

or

$$\eta = k_2 t. \quad (2)$$

Equation (1) applies if the liquid runs through the capillary under the influence of gravity alone, equation (2) if an applied pressure is used. Values of k_1 and k_2 shown in Table 2 were calculated from the times of flow given for water using values of η and ρ obtained from standard tables (Kaye & Laby (13)). As expected, the differences between corresponding

Table 2. *Values of experimental constants in equations* (1) *and* (2)

Temperature (° C.)	0	5	10	15	20	25	30
k_1 (c.g.s. units × 10^5)	2·47	2·40	2·39	2·35	2·69	2·28	2·22
k_2 (c.g.s. units × 10^5)	2·47	2·40	2·39	2·34	2·68	2·27	2·21

values of k_1 and k_2 are small, but neither is constant over the temperature range; both values show an approximately linear decrease with increase in temperature. This fact itself may reflect adversely on the validity of the experimental results as quoted, but apart from this the values of k_1 and k_2 at 20° C. are in each case decisively removed from the line through the remaining values. Accordingly, and considering that the behaviour and viscosity of water under such circumstances have been well established as free from such irregularities, it may reasonably be assumed that the above anomalous time of flow is not representative. It is interesting to speculate that perhaps the irregularity was attributed by Soxhlet to an excessive time of flow at 25° C. leading him to take a triplicate reading at that temperature. Soxhlet subsequently stated in the report that the increase of flow with temperature undergoes with water, and not milk, a small deviation between 15 and 25° C.

Omitting the value quoted for the time of flow of water at 20° C., an interpolated value was obtained giving a time of 438·0 sec. at 20° C. The relative time of flow now became 1·813 and the inversion was removed. The value thus obtained is shown plotted in Fig. 1. An apparent discrepancy at 10° C., at which temperature the relative viscosity shown is lower than might be expected from the other results, is due to a large time of flow for the milk. The difference is considered to lie within the experimental errors as shown by other times of flow, and in the absence of other evidence the value has been retained. It is to be noted that the figures given in Soxhlet's paper are in fact measures of the relative time of flow, and if equation (1), above, is appropriate, as seems likely, the figures would need multiplication by the values of the factor ρ_{water}/ρ_{milk} at each temperature to obtain, strictly, the relative viscosities.

Results derived from Soxhlet's figures have appeared in subsequent literature. In an attempt to transform the figures on to a scale giving to water at 20° C. a fluidity* of unity, the times of flow for milk have been divided by the time of flow for water at 20° C. Tables or values so calculated have been shown as the viscosity, and their reciprocals as the fluidity, in a number of standard text-books and in other literature, references (7) to (12). The figures obtained naturally lie on a smooth curve, this being, apart from a scale factor,

* Fluidity is defined as the reciprocal of viscosity; the viscosity of water at 20° C. is given in Kaye & Laby (13) as 0·01006 poise.

simply the curve for the times of flow of the milk at different temperatures. In the light of the preceding discussion on the dubiety of the time of flow for water at 20° C., however, these derived tables are not regarded as satisfactory. Amended values, using as the time of flow for water at 20° C. the interpolated value found above, are shown in Table 3 as relative rates of mean flow. They are not in fact values of the viscosity or relative viscosity according to the usual scientific definitions of these quantities.

Table 3. *Comparisons of the rate of flow of milk at different temperatures with the rate of flow of water at* 20° C.

Temperature (° C.)	Rate of flow of water at 20° C. / Rate of flow of milk	Rate of flow of milk / Rate of flow of water at 20° C.
0	3·69	0·271
5	3·02	0·331
10	2·41	0·415
15	2·11	0·473
20	1·83	0·548
25	1·59	0·628
30	1·41	0·711

It is also to be noted that in the paper by Spöttel & Gneist (5), Soxhlet's results have been tabulated as values of viscosity without clear indication that they refer to relative times of flow. In the same table a possibly not fortuitous inversion of the results for 10 and 20° C. avoids the irregularity at 20° C. previously discussed.

Kobler (2)

A horizontal viscometer attributed to Hess was used to compare the viscosity of milk with that of distilled water. Milks from different quarters of the same cow gave different viscosities, and accordingly a mixed sample was used at from 4 to 10 hr. after milking, this to avoid a false meniscus due to gas content. For the determinations at different temperatures samples were held for at least 1 hr. at the required temperature, and it was found that small temperature differences in the range 15–25° C. had little effect on the relative viscosity. It is stated that between 15 and 25° C. the results obtained were accurate to about 2 %, between 6 and 15° C. the accuracy was between 5 and 10 %, and in the latter case milk with the highest viscosity showed the greatest deviations. Eight samples were investigated at temperatures 6, 15 and 25° C., and to examine the reproducibility of results ten observations at short intervals were made on the viscosity of one milk and twelve observations on another. These latter results show no evidence of trends, and the two coefficients of variation are 0·5 % and 0·8 %. The figures for the viscosities of the eight milks at the three temperatures are shown below, in Table 4, and figures for sample 6 are shown in Fig. 1 in the Appendix.

Table 4. *Relative viscosities of eight milks at different temperatures* (*Kobler*)

Milk	Temperature (° C.) 6	15	25
1	2·30	2·08	2·02
2	2·02	1·86	1·82
3	1·89	1·79	1·75
4	1·96	1·82	1·79
5	1·88	1·76	1·72
6	1·94	1·82	1·78
7	1·81	1·73	1·69
8	1·83	1·74	1·70

Taylor (3)

Ostwald's modification of Poiseuille's apparatus was used for determinations of the relative viscosity as given, it is stated, by the product

$$\frac{t_{\text{milk}}}{t_{\text{water}}} \times \frac{\rho_{\text{water}}}{\rho_{\text{milk}}},$$

wherein, as before, ρ is the density and t the time of flow. The volume of each liquid used is not given, but the time of flow for water at 20° C. was about one-quarter of the corresponding time in Soxhlet's experiment. To avoid any appreciable decomposition, determinations were carried out within 4 hr. after milking. From the values of relative viscosity obtained at three temperatures for a single milk, constants were calculated stated to be those in Poiseuille's empirical formula

$$\eta_\theta = \frac{\eta_0}{1+\alpha\theta+\beta\theta^2}, \qquad (3)^*$$

where η_0 is the viscosity at 0° C. and η_θ the viscosity at θ° C. The formula obtained was then used to calculate the relative viscosities at 40° C. of a number of milks and the calculated values were compared with those observed. The results are shown in Tables 5 and 6 and in Fig. 1.

Table 5. *Relative viscosity determinations used for the formula*

Temperature (° C.) ...	20	25	30
Relative viscosity	2·100	2·034	1·987

Table 6. *Observed relative viscosities of ten milks at* 20 *and* 40° C. *and their calculated relative viscosities at* 40° C. (*Taylor*)

Milk	Relative viscosity at 20° C.	Relative viscosity at 40° C.	
	Observed	Observed	Calculated
1	2·006	1·853	1·853
2	2·029	1·870	1·875
3	1·951	1·800	1·802
4	1·984	1·834	1·833
5	1·992	1·845	1·840
6	2·032	1·878	1·878
7	1·953	1·812	1·805
8	1·997	1·844	1·845
9	1·966	1·823	1·817
10	1·961	1·812	1·812

The values of α and β calculated are given as

$$\alpha = 0{\cdot}00723,\ \beta = -0{\cdot}000156. \qquad (4)$$

It should, however, be observed that these values cannot be directly substituted in formula (3), even if the formula is taken as it stands to apply to relative viscosities. Instead, calculation shows that the above values of α and β are in fact relevant to the modified formula

$$\eta'_\theta = \frac{\eta'_{20}}{1+\alpha(\theta-20)+\beta(\theta-20)^2}. \qquad (5)$$

* Here and elsewhere the original notation has been altered for consistency.

This formula, in which η' denotes relative viscosity, connects the relative viscosity at $\theta°$ C. with η'_{20}, the relative viscosity at 20° C.

The maximum percentage difference between the observed and calculated relative viscosities shown in Table 6 is 0·4%, which represents very good agreement, considering that the values of α and β used were obtained from the results of a single milk sample.

Since formula (3) is usually quoted, it is not always clear in reports of Taylor's results in text-books and elsewhere that the values of α and β he obtained refer to formula (5). Apart from this, however, books and reports frequently show β with a positive sign. A positive value for β will not give agreement with Taylor's own figures, even using the modified formula.

Tapernoux & Vuillaume (4)

In this more recent experiment the absolute viscosity was determined using a modification of Ostwald's viscometer attributed to Baume. After milking, the milk was kept at a mean temperature of 1° C. until its examination 12–24 hr. later. In equation (1), k_1 was known, ρ was determined using a density balance and t was measured by a chronometer graduated in one-fifths of a second. It is stated that the error in viscosity, chiefly due to the time measurement, was less than 1·5 %. Although a number of milks were used to investigate the influences of other factors on the viscosity, the results on only one milk are given for the temperature effects. These results are shown in Table 7 and in Fig. 2 in the Appendix. No formula is given as a relation between viscosity and temperature, although a hyperbola is suggested for the curve.

Table 7. *Absolute viscosity at different temperatures* (*Tapernoux & Vuillaume*)

Temperature (° C.)	0	5	10	15	20	25	30	35	40
Viscosity (poise)	0·0344	0·0305	0·0264	0·0231	0·0199	0·0170	0·0149	0·0134	0·0123

Spöttel & Gneist (5)

A Höppler falling ball precision viscometer was used in this comprehensive experiment to obtain accurate measurements of the absolute viscosity in a temperature range of 15–45° C. The ball was machined to a diameter tolerance of ±0·0005 mm., the tube was sloped at an angle of 10·5° from the vertical and the ball was timed between marks 100 mm. apart; it reached terminal velocity before it had fallen to the upper mark. The tube was surrounded by an electrically heated and controlled water-bath which maintained the temperature constant to ±0·1° C. Three runs were made at each of the temperatures 15, 25, 35 and 45° C., and using the mean time at each temperature the absolute viscosity was obtained from the formula

$$\eta = kt(s_b - s_m), \tag{6}$$

where η is the absolute viscosity, t is the time of fall in seconds, s_b is the specific gravity of the ball and s_m the specific gravity of the milk; k is a constant for the particular experimental arrangements employed. The authors state that by preheating to between 40 and 50° C. and cooling to the desired temperature, the viscosity measured was equivalent to that which would have been obtained by observation on fresh milk. No change in viscosity was shown with repeated runs.

The results given by the authors are shown in Table 8 and in Fig. 2. The original

measurements were made at intervening temperatures because of some difficulty in obtaining a direct observation at 20° C. No formula is given for a relation between temperature and viscosity.

Table 8. *Absolute viscosity at different temperatures (Spöttel & Gneist)*

Temperature (° C.)	10	20	30	40	50
Viscosity (poise)	0·0243	0·0179	0·0137	0·0107	0·0090

Caffyn (6)

A capillary tube method was employed to determine the absolute viscosities of six samples of homogenized milk at temperatures in a range of 20–80° C. The time of flow t for a known volume of liquid V to pass through a measured capillary tube under an applied pressure p was obtained, and the viscosity η calculated from Poiseuille's equation

$$\eta = \frac{\pi R^4 \rho t}{8Vl} - \frac{m\rho V}{8\pi l t}. \qquad (7)$$

In the formula R and l are the radius and length respectively of the capillary tube, ρ is the density of the milk and m a constant. The kinetic energy correction, the second term, was minimized by making t large so that the correction to the viscosity was of the order of 0·1% at 20° C., increasing to about 1% at the highest temperatures. All measurements were taken to a high degree of accuracy, corrections being made where necessary, and it is stated that the viscosity-temperature coefficient was determined with an estimated accuracy of 1%. The homogenized milk was stored at 0° C. before being placed in the viscometer and runs began about 10 min. after this time. Determinations of composition were obtained for the milks used, and their densities were found from standard tables. Investigations were also carried out on a rise in viscosity observed when repeated runs were made. The observations on values of the viscosity at different temperatures are given in Table 9 and the observations for sample 4 are shown in Fig. 2.

Table 9. *Absolute viscosities (η) at different temperatures (θ) of six homogenized milk samples (Caffyn)*

(η in poise, θ in ° C.)

Sample number												
1		2		3		4		5		6		
θ	η	θ	η	θ	η	θ	η	θ	η	θ	η	
19·6	0·0198	20·1	0·0199	19·9	0·0206	22·1	0·0183	20·2	0·0201	20·6	0·0192	
30·0	0·0147	29·9	0·0153	30·5	0·0150	30·6	0·0148	28·8	0·0152	32·9	0·0136	
40·7	0·0111	41·9	0·0113	39·8	0·0119	39·6	0·0116	40·7	0·0113	39·3	0·0118	
49·9	0·0092	49·0	0·0098	49·5	0·0098	49·7	0·0097	48·2	0·0096	51·3	0·0092	
60·2	0·0076	58·9	0·0082	60·1	0·0085	59·0	0·0086	59·3	0·0079	67·8	0·0070	
70·0	0·0066	68·9	0·0070	70·5	0·0071	69·3	0·0077	69·6	0·0068		—	
78·1	0·0060	79·6	0·0063	79·7	0·0064	80·3	0·0061	79·1	0·0061		—	

3. DISCUSSION AND RESULTS

A general discussion on the viscosity of colloidal solutions may be found in Hatschek (14), and a comprehensive review of the work on milk and its products has been made by Scott-Blair (15). Attention is here confined to the viscosity of whole milk, with particular reference to the results already quoted.

With such a complex product as whole milk it is not to be expected that an observed physical property will behave accurately according to a simple fundamental law. And although some of the individual constituents affecting the viscosity of milk may have comparatively simple viscosity-temperature relations, it is not likely that these will combine in such a way that the resultant relation is correspondingly simple. In fact, the viscosity observed on any occasion may be regarded as an 'effective' or 'equivalent' viscosity and one depending, therefore, on the particular experimental circumstances attending the observation. A falling ball viscometer, for example, will give a different integration of micro-viscous contributions than will a time-of-flow apparatus. And since water is the predominant constituent of milk, the density and, correspondingly, the viscosity of milk may increase with time at the higher temperatures due to evaporation; it may be that this effect makes some contribution to the increase in viscosity observed (Caffyn (6)) when repeated runs are made. Differences in state and, for example, in the composition of the milk under examination may also be effective, and ideally a complete specification including an analysis of composition would accompany any determination of the viscosity. Observations on the effects of composition alone were made in some of the preceding experiments, and although it seems probable that variations in composition would have only secondary effects on the changes of viscosity with temperature, direct experimentation would be needed to obtain reliable information on this and similar questions. Caffyn (6) has also pointed out, with reference to capillary tube determinations, the possibility that creaming may occur during the course of a single run, leading to an error in the value of the viscosity. It is to be noted, however, that where repeated observations were made, in the experiments of Soxhlet (1) and Spöttel & Gneist (5), good agreement was obtained between replicates, nor were trends observed such as those noted by Caffyn. The possibility nevertheless remains that an effect such as creaming may develop (so that the process of observation itself changes the quantity observed), but it is considered preferable not to regard this as a source of error but rather as an admissible contribution to the 'effective viscosity' discussed above. Similarly, the effects of melting fat (Caffyn (6)) may be regarded as admissible under the defined experimental circumstances.

From the above remarks it is considered that no general relation independent of experimental circumstances can reasonably be expected to describe with precision the changes with temperature in the viscosity of whole milk. Accordingly, it may be observed that although the experiments discussed above gave results which showed good internal agreement, comparison of the results of the different experiments showed less consistency. Attempts to compare the results of the experiments by conversion of all observations to either relative or absolute viscosity gave little improvement.

Empirical curves have therefore been fitted by the method of least squares to the results of the experiments discussed above. The observations of Caffyn (6) show that the logarithm of the viscosity can only be regarded as linearly related to the reciprocal of the absolute temperature over a limited range and no other simple transformation was found which would enable a straight line to be fitted to the data. Simple polynomial expressions have accordingly been used to connect the viscosity or relative viscosity at another temperature with values at 20° C.

The results for relative viscosity are shown in Table 10, as coefficients in the equation

$$\eta'_\theta - \eta'_{20} = a_1(\theta - 20) + a_2(\theta - 20)^2, \qquad (8)$$

where η'_θ is the relative viscosity at θ° C.

Table 10. *Coefficients in equations showing the changes with temperature in the relative viscosity of whole milk*

Source of data	$a_1 \times 10^3$	$a_2 \times 10^4$
Soxhlet		
1*	−12·56	3·81
Kobler		
1	− 6·00	9·71
2	− 4·00	7·25
3	− 4·00	3·74
4	− 3·00	6·61
5	− 4·00	4·91
6	− 4·00	4·91
7	− 4·00	2·57
8	− 4·00	3·16
1–8 combined	− 4·13	5·36
Taylor		
1	−15·10	3·80

* Values refer to relative time of flow.

The curves so obtained are shown fitting the observed results in Fig. 1 in the Appendix, where for clarity the curve derived from the combined observations is shown for the results of sample 6 in Kobler's experiment. The individual milks examined by Kobler give similar curves, and although different from the general form fitting Kobler's results, the curves for the milks examined by Soxhlet and Taylor are in themselves similar.

A quadratic curve is of course sufficient to give an exact fit to three points as in the case of the observations made by Taylor and Kobler on individual milks. The single curve obtained from the combined observations in Kobler's experiment gives, however, a very reasonable fit for the individual milks themselves. The worst fit occurred with the first milk, for which the percentage deviations at temperatures 6, 15 and 25° C. were respectively −3, +1 and −2%. For the other milks the deviations were generally of the order of 1%. The maximum deviation from the curve fitted to the observations made by Soxhlet on relative time of flow was 2·8% at 10° C., ignoring the anomalous value at 20° C.

For the observations on absolute viscosity a cubic term was found necessary to obtain a fit subject to the experimental variability, and the equation of the curve now becomes

$$\eta_\theta - \eta_{20} = b_1(\theta-20) + b_2(\theta-20)^2 + b_3(\theta-20)^3, \qquad (9)$$

where η_θ is the absolute viscosity in poises, at θ° C. The values of b_1, b_2 and b_3 obtained from the results of the experiments examined are shown in Table 11, and the curves so

Table 11. *Coefficients in equations showing the changes with temperature in the absolute viscosity of whole milk*

Source of data	$b_1 \times 10^4$	$b_2 \times 10^6$	$b_3 \times 10^8$
Tapernoux and Vuillaume			
1	−5·919	8·866	9·899
Spöttel & Gneist			
1	−5·230	9·821	−7·500
Caffyn*			
1	−5·680	8·954	−5·513
2	−5·423	7·815	−4·303
3	−6·331	11·706	−8·645
4	−5·837	11·000	−8·315
5	−6·237	10·811	−7·260
6	−5·746	10·326	−7·932
1–6 combined	−5·895	10·089	−6·886

* Homogenized milk used.

obtained are shown in Fig. 2 in the Appendix. A single curve from the combined observations is shown fitting sample 4 for the homogenized milks.

It may be seen that all the curves are of the same general form, and no major differences are apparent between the observed viscosity-temperature relations of the whole milks used in the first two experiments and the observed viscosity-temperature relations of the homogenized milk used by Caffyn.

The maximum deviations of the observed figures from the curves fitted to the results from individual milks were for the milk examined by Tapernoux & Vuillaume, 0·75% at 35° C., and for the milk examined by Spöttel & Gneist, 0·66% at 30° C. The deviations in the case of the milks examined by Caffyn were slightly larger, the maximum deviation being 2·2% at 70·5° C., for the third sample. The curve obtained from the combined observations of Caffyn's experiment gives a reasonable fit to the observations for the individual milks; for samples 1, 5 and 6 the deviations were of the order of 2% throughout the range of temperatures investigated and for samples 2–4 there were maximum deviations of 4·0% at 68·9° C., 5·8% at 69·3° C. and 4·5% at 79·7° C., respectively.

The accuracy obtained by using the appropriate formula in practice would, in general, be slightly less than that indicated by the figures above. It would depend on the accuracy of the determination which needs to be made at a convenient temperature to position the curve.

SUMMARY

1. The results of experiments on the changes with temperature in the relative viscosity and viscosity of whole milk have been considered.
2. Some inconsistencies have been traced and amendments proposed.
3. It has been suggested that values for the relative viscosity and viscosity of whole milk cannot usefully be regarded as independent of the experimental circumstances in which the observations were obtained.
4. Empirical curves have been calculated for the viscosity-temperature relations, in forms convenient for practical use in circumstances similar to those of the experiments examined.

Grateful acknowledgement is here made to Mr N. de Vicq for translations of the relevant parts of the German papers and to Mr J. E. Caffyn for the figures obtained in his experiment. I wish also to thank Dr G. W. Scott-Blair for drawing my attention to the work of Mr Caffyn, and all members of my staff for their assistance with calculations and graphs.

APPENDIX

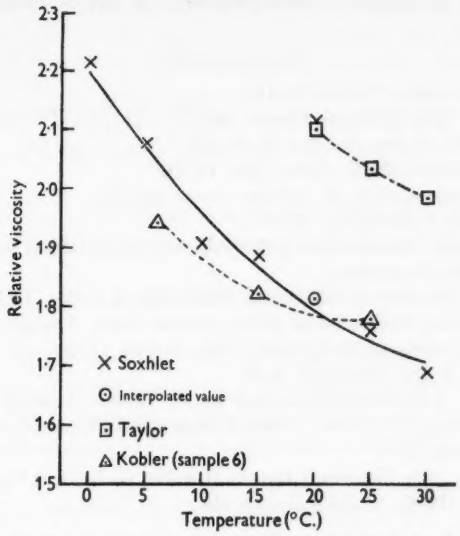

Fig. 1. Relations between temperature and the relative viscosity of whole milk.

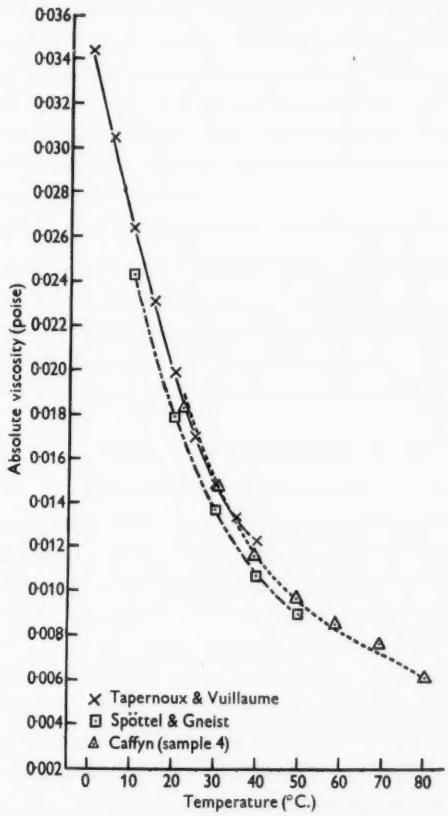

Fig. 2. Relations between temperature and the absolute viscosity of whole milk.

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637.127.2

464. A SIMPLE METHOD FOR THE ROUTINE DETERMINATION OF LACTOSE IN MILK*

By F. J. FRANCIS† and M. DOREEN SMITH
Department of Food Chemistry, University of Toronto, Toronto, Canada

(With 2 Figures)

The estimation of the lactose content of milk is useful in the milk processing industry as well as in dairy research. A study was made of the analytical methods used for lactose in an attempt to evolve a more satisfactory procedure. To this end, the method of Hassid (8) for the determination of reducing sugars in plants was examined with a view to adapting it to lactose.

The method of Hassid, which might be termed the ferricyanide-cerimetric method for the estimation of reducing sugars, is based on the oxidation of the sugar with alkaline potassium ferricyanide solution and the titration of the ferrocyanide formed with sulphato-ceric acid. The use of the cerium salt was introduced by Whitmoyer (21), and the method so modified has been applied to a variety of carbohydrate compounds such as invert sugar (21), sugar in plant extracts (20), in root extracts (1), in blood (17, 7, 11), in potatoes (22), in enzyme digestions (9, 10), fructose (17), and glucuronic acid (5, 14) and starch (15). The use of salts of cerium is well established in the fields of organic and inorganic analysis (2). The reaction of the sulphato-ceric acid with the potassium ferrocyanide formed by reacting with a sugar, may be expressed as follows:

$$2K_4Fe(CN)_6 + 2H_4Ce(SO_4)_4 \rightarrow 2K_3Fe(CN)_6 + Ce(SO_4)_3 + 2KHSO_4 + 3H_2SO_4.$$

In this report the conditions are established for the application of the ferricyanide-cerimetric procedure to routine determinations of lactose in milk or for the estimation of small quantities of lactose. It is simple and takes considerably less time than the methods now in use. The values were higher than those obtained by the method of the Association of Official Agricultural Chemists (19) or by the Lane & Eynon technique (13).

DEVELOPMENT OF METHOD

The conditions of the reaction

When lactose was substituted for the glucose in the ferricyanide-cerimetric procedure as used by Hassid (8), preliminary experiments showed that certain conditions of the reaction had to be established. The concentration of sodium carbonate in the reaction tubes should be at least 0·1%. The curve obtained by plotting lactose oxidized against pH is linear with slope 3·0 up to pH 9·95. Above this value the curve levels off; thus the amount of sodium carbonate should be sufficient to give a pH value of 10–11. A 15 min. reaction time should be adhered to as an increase in time will increase slightly the sulphato-ceric acid titre.

Lampitt, Fuller, Goldenburg & Green (12) stated that the reducing power of dextrose and maltose in a carbonate-ferricyanide oxidation mixture increases in the presence of sodium

* Aided in part by a grant from the Advisory Committee on Scientific Research, University of Toronto.
† Now at Ontario Agricultural College, Guelph, Ontario.

chloride. Experiments with lactose plus the normal amount of sodium chloride found in milk (0·10 %) and five times the normal amount did not appreciably change the titration values. Therefore, it appears that the presence of chlorides in the milk does not need to be taken into account.

The amount of sulphato-ceric acid consumed with a given amount of lactose varies with the ratio of ferricyanide to lactose. The graph of ml. of 0·01 N-sulphato-ceric acid utilized, plotted against the ferricyanide-lactose ratio, is linear from ratio 1 : 1 to 6·5 : 1 with slope equal to 0·6 (Fig. 1). Above the ratio 6·5 : 1, the graph is again linear with slope

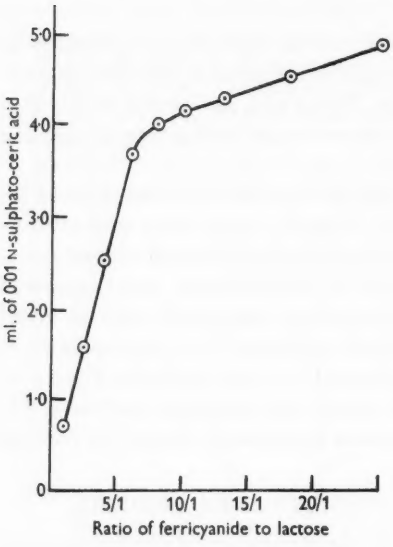

Fig 1.

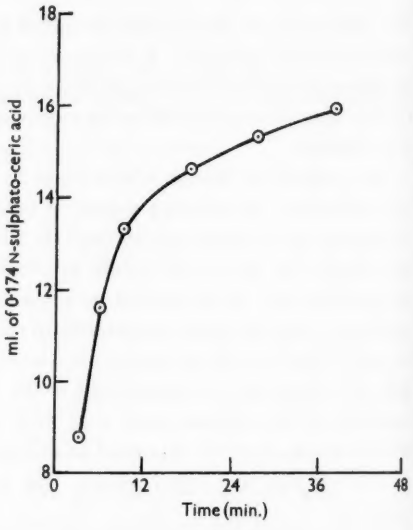

Fig. 2.

Fig. 1. Graph to show how ratio of ferricyanide to lactose affects the sulphato-ceric acid titre. Amount of lactose is constant (2 mg.).

Fig. 2. Graph to show how amount of sulphato-ceric acid consumed increases with time. Amount of lactose is constant (10 mg.).

equal to 0·02. The fact that the graph in the higher ratios does not have a slope equal to zero means that the extent of the reaction between the lactose and the ferricyanide depends on the ratio between the two. Thus, in analyses for lactose using this method, it is not possible to use a relationship such as 1 mg. of sugar = 3·00 ml. of 0·01 N-sulphato-ceric acid as Hassid (6) suggests for glucose. In the concentrations specified under 'Reagents', the graph of sulphato-ceric acid against lactose is linear over a range of 0–7 g. of lactose per 100 ml. of milk.

The possibility of increasing the accuracy of the method by increasing the reagent concentrations was investigated. If the millilitres of sulphato-ceric acid consumed be plotted against time, with the amount of lactose constant, the curve is steep at first and then changes its slope (Fig. 2). The latter part of the curve (i.e. after the main reaction has occurred) is not flatter with increased reagent concentrations; thus any increase in accuracy is doubtful.

Table 1. *Effect of different clarifying agents on apparent lactose content of milk*

Sample	Clarifier	Percentage lactose (w/v)		Percentage difference
		Lane & Eynon method	Ferricyanide-cerimetric method	
1	Dialysed iron	4·69*	4·79	2·09
1	,,	4·69*	4·80	2·29
2	,,	4·69*	4·76	1·47
2	,,	4·69*	4·76	1·47
3	Farmer's reagent	4·34	4·67	7·06
3	,,	4·34	4·66	6·87
4	,,	4·24	4·40	3·64
5	,,	4·42	4·87	9·24
6	Zinc hydroxide	4·60*	4·75	3·16
6	,,	4·60*	4·75	3·16
7	,,	4·62*	4·77	3·15
7	,,	4·62*	4·78	3·14
8	Cadmium hydroxide	4·34*	4·82	9·95
8	,,	4·33*	4·79	9·61
9	,,	4·38*	4·85	9·70
9	,,	4·38*	4·85	9·70

* Decalcified with oxalate after filtering off precipitate of protein.

Clarifying agents

A series of clarifying agents for milk were tested with the ferricyanide-cerimetric method, and the results were compared with the volumetric Lane & Eynon method (13). The lead acetate-sodium fluoride reagent, known as 'Farmer's Reagent' (3), is an excellent clarifier for the Lane & Eynon method as it removes both protein and calcium. It is, however, unsuitable for the ferricyanide-cerimetric method as lead, fluoride and oxalate were all found to lower the sulphato-ceric acid titration values. Several workers (13, 18) have reported that calcium inhibits the reduction of copper by lactose resulting in apparently lower contents of lactose. Calcium was found to have no effect in the ferricyanide-cerimetric method.

Cadmium hydroxide, introduced by McDowell (16) as a clarifier for milk, gave results with the ferricyanide-cerimetric method approximately 9 % higher than the Lane & Eynon method. Zinc hydroxide (6) gave values approximately 3 % higher.

Dialysed (colloidal) iron, introduced by Fine (4), is a very effective clarifier for milk. Analyses on filtrates of milk clarified with dialysed iron gave values for iron content similar to those for whole milk, indicating that all the colloidal iron added is precipitated. This clarifying agent was adopted in the following method.

DESCRIPTION OF RECOMMENDED METHOD FOR DETERMINATION OF LACTOSE IN MILK. REAGENTS

(1) Alkaline potassium ferricyanide reagent. 8 g. of potassium ferricyanide and 10 g. of sodium carbonate made up to 1 l.

(2) Dialysed iron (5 % Fe_2O_3).

(3) Setopaline C indicator solution: 0·1 % (w/v). (May be obtained from Eimer and Amend, New York, N.Y.)

(4) Stock lactose solution: 1 % monohydrate (w/v).

(5) Sulphato-ceric acid: 0·01 N. 5·4826 g. of ammonium hexanitrato-cerate

$((NH_4)_2Ce(NO_3)_6)$* were placed in a beaker and stirred with 28 ml. of concentrated sulphuric acid. Distilled water was added carefully in 100 ml. lots and the volume made up to 1 l. Otherwise the solution may precipitate an appreciable amount of insoluble material (2). (The purpose in preparing the reagent in this manner is to convert the nitrato-cerate to a form of sulphato-cerate.)

(6) Sulphuric acid: 5 N.

METHOD

The calibration between millilitres of sulphato-ceric acid and milligrams of lactose must be established. Pipette 5 ml. of lactose solution containing 1 mg. of lactose (stock solution diluted 1 in 50) into each of three test-tubes (150 × 25 mm.). Similarly, prepare three tubes each containing 2 mg. of lactose in 5 ml. of solution. Add 5 ml. of alkaline ferricyanide solution to each of the six tubes and place in a boiling-water bath for exactly 15 min. Remove the tubes from the bath, cool to room temperature by immersion in running water for 3 min., and add 5 ml. of 5 N-sulphuric acid solution. Add eight drops of setopaline C indicator and titrate with sulphato-ceric acid solution. The colour change for the indicator is green to golden brown. The graph of millilitres of 0·0100 N-sulphato-ceric acid (uncorrected for titration blanks) against milligrams of lactose monohydrate has a slope of 2·22.

Pipette 10 ml. of milk into a 250 ml. volumetric flask containing 100 ml. of distilled water. Add 7 ml. of dialysed iron slowly with shaking and make up to volume. Allow the coarse brown precipitate to settle and filter. Dilute the filtrate 1 in 10 and add 5 ml. of the diluted filtrate to each of two test-tubes. Add the ferricyanide solution, heat, cool, add sulphuric acid and titrate with sulphato-ceric acid as outlined above. From the calibration curve, read the milligrams of lactose corresponding to the sulphato-ceric acid titre. This value, multiplied by 2·5, gives the grams of lactose in 100 ml. of milk.

ACCURACY OF THE METHOD

Comparison with the official method of the Association of Official Agricultural Chemists

The ferricyanide-cerimetric procedure suggested under 'Method' was compared with the official A.O.A.C. gravimetric copper reduction method (19). The results are expressed in Table 2.

Table 2. *Comparison of official A.O.A.C. method and the ferricyanide-cerimetric method for the estimation of lactose in milk*

	Percentage lactose (w/v)		
Sample	A.O.A.C. method	Ferr.-cer. method	Percentage difference
10	4·51	4·66	3·3
11	4·52	4·67	3·2
12	4·57	4·74	3·5
13	4·56	4·74	3·8

The proposed method gave results approximately 3·5 % higher than the official A.O.A.C. method and 2 % higher than the Lane & Eynon method (Table 1). These results are considered satisfactory, since McDowell (16) has pointed out that the copper reduction method

* May be obtained from the G. Frederick Smith Chem. Co., Columbus, Ohio.

gives results which are 0·2–0·4% too low as compared with polarimetric, iodimetric and chloramine T methods. He states that the apparent lactose content may vary as much as 5% depending on the method and clarifying agent used.

RECOVERY EXPERIMENTS

The results of determinations on milk with added lactose are presented in Table 3. Duplicate analyses are easily reproducible to within 0·5%.

Table 3. *Recovery experiments with the ferricyanide-cerimetric method*

Sample of milk	No lactose added	One g. of lactose added per 100 ml. of milk	Two g. of lactose added per 100 ml.
	Percentage lactose found		
14	4·75	5·73	6·75
15	4·75	5·76	6·76
16	4·83	5·85	6·83

NOTE

Since this work was completed, Williams (22) has reported results on the application of the ferricyanide-cerimetric method to the determination of sugar in potatoes. He found that the use of ion-exchange resins would remove the non-sugar reducing compounds which amounted to as high as 30% of the apparent sugar values. Inclusion of an ion-exchange step may improve this method, if the increased accuracy is warranted.

SUMMARY

A method for the determination of lactose in milk based on clarification with dialysed iron and oxidation of the filtrate with alkaline ferricyanide solution, followed by titration with sulphato-ceric acid, is proposed. It is simple, rapid and well adapted to the routine estimation of lactose in milk or to the determination of small quantities of lactose. The conditions of the reactions and comparison of results with other methods are discussed.

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465. STUDIES ON VARIATIONS IN THE SOLIDS-NOT-FAT CONTENT OF MILK

I. VARIATIONS IN LACTATION YIELD, MILK-FAT PERCENTAGE AND SOLIDS-NOT-FAT PERCENTAGE, THAT ARE DUE TO THE AGE OF THE COW

By G. L. BAILEY
National Institute for Research in Dairying, University of Reading

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FOREWORD

The known causes of variation in milk quality fall into two groups, those that depend on the character of the individual cow, and those that depend on external factors. The first group of factors causing variation include breed and strain within breed, age of cow and stage in lactation. The farmer cannot control these factors save in the long term; but they have such a powerful influence on milk quality that all progressive farmers arrange their herd management and direct its breeding so that these factors operate to the best advantage.

The factors of the second group that influence the quality of milk all have a short-term effect in that the effect starts and stops with the application and removal of the factor; perhaps the most influential and easily controlled of these is the plane of nutrition of the cow, but under expert management the use of certain hormones such as L-thyroxine or oestrogens can bring about drastic changes in milk composition. Other known factors in this group include disease and its cure, time of day for milking, milking interval, completeness of stripping and sometimes air temperature, although this is seldom important in this country.

In spite of all the information available on variations in milk quality, changes in quality sometimes occur that are very difficult to attribute to any one of the known factors. Two such changes have been reported in recent years. First, Rowland (1, 2) showed that an increasing number of whole herds of cows had been producing milk low in

the solids-not-fat fraction during the late winter months; secondly, Provan & Jenkins (3) showed that during the last two decades the percentage of solids-not-fat had fallen for a considerable proportion of the nation's cows. In both these reports expert judgement was given on the probable causes of the trends, but owing to the limitations of the data used to demonstrate the trends a completely satisfying estimate of the importance of each of the probable factors was not possible.

Consideration of the factors known to influence the quality of milk shows that very full records of the management, character and milk quality of many individual cows are necessary if a statistical evaluation of the factors involved in a particular trend is to be attempted.

Many investigations of milk quality, including the two referred to, have been made with the help of records of milk quality obtained in the laboratories of milk factories. These refer to the composition of bulked milks and under such conditions details of individual cows cannot be known; therefore the terms necessary for a mathematical solution cannot be met. Unfortunately, milk factories are almost the only source of continuous records of the solids-not-fat fraction of milk, for there is no nation-wide scheme for testing this fraction similar to the readily available butter-fat testing service of the Milk Marketing Board. The estimations of the solids-not-fat fraction that have been made for research purposes have seldom been made over an extended period of time, and indeed the only records known to the author, that have been kept for this fraction of milk for individual cows and which are accompanied by faithfully kept records of cow management, are those accumulated for the herd of Dairy Shorthorn cows maintained at Shinfield by the National Institute for Research in Dairying.

Compared with investigations using the extensive records of milk factories, an investigation using the records of only one herd of cows must suffer from the usual disadvantage experienced when only small numbers are studied; only large variations in milk quality can be judged significant, but it is considered that this drawback is more than counterbalanced by the advantage of homogeneity and lack of bias in the estimation of the importance of a particular factor.

The results of a series of investigations carried out on the records of the herd of Dairy Shorthorn cows, managed by the N.I.R.D. are reported here; in most cases the studies have been made on records accumulated during the twelve years from 1935 to 1946.

The breeding and management of the cows

The herd of Dairy Shorthorn cows maintained by the National Institute for Research in Dairying has been built up from animals purchased during the years 1920–4. At first, no pedigree stock were bought in, but by 1924 there were five out of a total of eighty-four animals that were entered in Coates's herd book. A policy of grading up was inaugurated, and by 1946 the herd consisted of eighty-eight pedigree animals with a further twelve still in the grading-up process. The bulls used have not been high-priced animals, but care has been taken to ensure good type, and the animals related to the bulls have been examined to ensure that the stock was of uniform quality.

The herd has existed primarily to provide milk and animals for research purposes, but subject to this the aims of the Institute in forming the herd were tabulated by Mackintosh (4) in a short history of the herd's early years. These aims were to build up

a home-bred herd, with good milking qualities, of good breed type, with good udders and free from tuberculosis. The pursuit of each of these ideals has not been attended with equal success; perhaps the most serious of the difficulties encountered have been two outbreaks of contagious abortion. The first of these occurred in 1934, and an attempt was made to eradicate the disease by segregation and disposal of reactors, but this policy had to be abandoned owing to the scale of the outbreak; eighteen animals had been sold before the policy was given up. The second outbreak occurred in 1940, chiefly among the first calf heifers. Since 1944 all calves, heifers and cows have been vaccinated to prevent further outbreaks.

The continuity of the herd breeding policy was seriously interrupted by these outbreaks; the loss of the young animals and the disposal of many of the breeding stock led to a marked decline in herd quality, a trend that was clearly demonstrated by the lower average for the herd milk yield in the years that followed the outbreaks (see Table 1). An investigation into the general fertility of the herd has been made by Foot & Ridler (5), and of calf

Table 1. *Average milk yields of N.I.R.D. herd of Dairy Shorthorn cows*

Year	No. of cows	Average yield (lb./lactation)	Average butter-fat (%)
1921–2	7	7158	—
1922–3	11	5159	—
1923–4	16	6986	—
1924–5	21	6976	3·76
1925–6	19	7291	3·79
1926–7	24	8549	3·71
1927–8	22	8820	3·78
1928–9	24	9474	3·84
1929–30	31	8305	3·68
1930–1	31	8371	3·76
1931–2	26	9117	3·69
1932–3*	32	7572	3·76
1933–4	25	7355	3·57
1934–5	21	6757	3·64
1935–6	21	6425	3·65
1936–7	28	6201	3·47
1937–8	30	7295	3·69
1938–9	31	7137	3·67
1939–40	32	6618	3·76
1940–1	22	7603	3·67
1941–2	31	6854	3·87
1942–3	39	6399	3·95
1943–4	37	7148	3·87
1944–5	36	7427	3·83
1945–6	28	7258	3·77

* From about this time the herd was used for a far larger number of experiments.

mortality, weight, and body measurements by Braude & Walker (6). There has been little trouble from tuberculosis, and only a few animals have reacted to the tuberculin test and have had to be sold.

The management of the herd has been on lines similar to those practised with commercial herds of similar type and size. The animals have not enjoyed any additional foods not available to other herds and, consequently, during the war years a high proportion of their food was home grown. During the early years of the herd's life, efforts were made to increase the milk yield by an excessively high level of feeding and much food was

wasted; this was soon shown to be uneconomical and was given up. Food rationing was then introduced and is now on the basis of 6 lb. starch equivalent and $\frac{6}{10}$ lb. protein equivalent for maintenance, and $2\frac{1}{2}$ lb. starch equivalent with $\frac{1}{2}$ lb. protein equivalent for each gallon of milk. Before 1938 the production protein equivalent ration was $\frac{6}{10}$ lb. for each gallon of milk. The production ration has been adjusted each week according to the individual cow's current milk yield. No allowance has been made in the ration for either the body weight of the cow or for the quality of milk.

Experiments with milking machines were a feature of many research projects for many years, but the entire herd was not so milked until 1946. Morning milking has started at 6.45 and afternoon milking at 3.45; very few cows and no first calvers have been milked more than twice in the day.

Most of the experiments have been carried out in the winter months when indoor feeding gives the most uniform conditions of management. The terms of the experimental management have seldom been outside the range of normal practice, but the changes in the cows' routines may well have exerted a depressing effect on their performances.

Herd records

Breeding records have been kept with very full details for all the animals that have been in the herd since its establishment. The particulars recorded include sires, dams and sex of calves, together with dates of births, deaths or disposals, services, calvings or abortions and dates of dryings off.

Feeding records have been kept for each cow separately. During the grazing season only the description and quantities of the supplementary foods have been recorded, but at other times details of the full concentrate and roughage rations are available. In contrast to the concentrate ration which has been carefully weighed out for each cow, the roughage ration has been judged by eye only, but the resultant error due to this is considered to be quite small, partly owing to the experience of the stockmen in estimating the weights of rough foods and partly to the conviction that errors would have occurred both above and below the correct level and can, over a period, be ignored.

In the case of milking records the milk yield of each cow has been weighed and recorded at each milking; weighings have been correct to the nearest quarter pound.

Milk samples for testing milk quality have been taken once a month for each cow while she has been in milk; the number of samples taken to obtain a true representation of the cow's milk has varied through the years, but all methods of sampling have involved two samples, an evening sample followed by another from the next morning's milk. The evening and morning milks have been analysed separately. The milk-fat fraction has been estimated by the Gerber method (British Standards Institution, 1936), the technique of which has not varied since the tests were started. Two methods of estimating the total solids of the milk have been employed: at first a lactometer method based on Richmond's formula was used, but Bartlett, Golding & Wagstaff(7) showed that this method was liable to give results that were too high for samples taken during the winter months. To correct this the method was changed, and since 1935 the total solids have been estimated by evaporation of the milk at 100° C. in an oven(8); further differences between the two methods of estimation have been reported by Boder & Campbell(9). The solids-not-fat fraction has been obtained by difference (solids-not-fat = total solids − milk fat) and has,

since 1934, been adjusted to allow for variations in the milk-fat fraction (10). The effect of the adjustment is shown in the sample calculation:

$$\text{Total solids} = 12\%, \text{ milk fat} = 3{\cdot}5\%,$$

$$\text{Solids-not-fat } \% = \frac{(12-3{\cdot}5)\,100}{100-3{\cdot}5} = 8{\cdot}81\%.$$

All figures of solids-not-fat in this work refer to the adjusted figures.

INTRODUCTION

Lactation records are the most suitable measurements to use to show the difference between cows of different breeds or strains within breed or between cows of different ages; of these factors this work is not concerned with breed differences as the records used have been gathered for cows of the Shorthorn breed only. Differences between strains within breed have not been assessed as these are of interest to a student of a particular breeding policy only; consequently, the effect of age differences alone have been studied in this part.

It is very well known that both milk yield and milk quality vary with the age of the cow; indeed so many studies have been made on the subject that some apology is called for when presenting yet another.

The study of this section was carried out primarily to provide detailed information for the planning of further survey work. In a study of cows of only one herd there is continual doubt as to the sufficiency of the number of figures available to reveal with high statistical significance small differences between mean values. To make the best use of the data available, figures that are representative, yet of comparatively uniform value, have to be selected from the main mass of data; classification by age groups provides a good opportunity to do this, for it is to be expected that the variation of the records of cows of only one age group will be less than the variation of the records of cows of all ages. The results of the study of this section provide good evidence for deciding which age group is the most suitable for further studies.

This study was also undertaken for the value of the data on solids-not-fat. So far as is known, partial correlation coefficients involving this fraction of milk and milk fat and milk yield have not been calculated before.

STATISTICAL METHODS

All the three lactation records, milk-yield, milk-fat and solids-not-fat percentages are to some extent dependent for their value on the duration of the lactation. This in turn depends in part on the state of health of the animal. Unhealthy animals have not been considered in this study and to eliminate them, lactations that ran for either a very long or a very short period, which are usually made by animals that are in some way diseased, have been omitted or modified. It is well known that mastitis and contagious abortion frequently lead to short lactations, while many very long lactations are the result of one of the several conditions that give rise to infertility or sterility; a barren animal or one that turns to its first service after calving is naturally kept in milk as long as possible. All lactations that lasted less than 200 days were omitted from the study; those that lasted for longer than 315 days were assumed to have ended at that time, and the records calculated so that all production after the 315th day was ignored; lactations that lasted between 200 and 315 days were assumed normal, so the records were accepted unaltered.

Lactation records of milk yield, measured in pounds, and milk fat and solids-not-fat, both estimated as percentages, were collected for all the cows that had calved during the years 1935–46; these were grouped according to the number of the lactation. All first calvers were grouped together and so on for each of the age groups up to the seventh; only five cows completed their eighth and three cows their ninth lactations, so these age groups were numerically too small to warrant separate study.

The groups of figures representing the first, second, third and fourth calvers were subclassified so that all the animals in one group that calved in any one month of the year were tabulated together. The groups of figures for the fifth, sixth and seventh calvers were not so treated, as there were too few figures in a group to warrant such a division. Mean figures for each of the groups and subgroups were calculated; the lactation means according to month of calving are presented in Table 3 and the lactation means disregarding month of calving in Table 2.

Table 2. *Average milk yield, milk-fat and solids-not-fat percentages for different lactation age groups of cows*

	First lactations	Second lactations	Third lactations	Fourth lactations	Fifth lactations	Sixth lactations	Seventh lactations
No. of Cows	137	97	79	52	31	16	11
Average milk yield (lb./lactation)	6437	7145	7765	8164	8008	8524	8358
Coefficient of variation (%)	19·67	28·23	25·20	20·94	26·40	19·60	30·90
Average milk fat (%)	3·83	3·77	3·68	3·73	3·68	3·57	3·65
Coefficient of variation (%)	7·92	8·66	12·26	8·60	23·44	6·85	4·20
Average S.N.F. (%)	9·31	9·21	9·15	9·11	8·95	8·86	8·94
Coefficient of variation (%)	2·84	2·91	3·07	3·14	3·32	3·37	2·90

Table 3. *Seasonal variations in lactation milk yield, lactation milk-fat percentage and lactation solids-not-fat percentage*

	First lactations				Second lactations			
Month of calving	No.	Average milk yield (lb./lactation)	Average milk fat (%)	Average S.N.F. (%)	No.	Average milk yield (lb./lactation)	Average milk fat (%)	Average S.N.F. (%)
Jan.	6	6994	3·68	9·15	8	7069	3·78	9·28
Feb.	9	7400	3·64	9·27	6	6825	3·67	9·19
Mar.	19	6304	3·85	9·33	9	7411	3·65	9·10
Apr.	7	7309	3·62	9·35	8	8707	3·57	9·06
May	11	5960	3·76	9·28	8	8076	3·63	9·04
June	7	5140	4·05	9·47	5	7659	3·66	9·16
July	0	—	—	—	3	7023	4·01	9·27
Aug.	14	6422	3·89	9·29	11	6562	3·83	9·50
Sept.	19	6347	3·83	9·28	12	6701	3·87	9·20
Oct.	14	5952	3·87	9·29	9	6530	3·98	9·30
Nov.	18	6711	3·78	9·36	9	7263	3·80	9·21
Dec.	13	6437	3·74	9·36	9	6643	3·70	9·17
	Third lactations				Fourth lactations			
Jan.	8	7558	3·75	9·15	5	9085	3·65	9·02
Feb.	4	9416	3·73	9·02	4	8721	3·83	9·20
Mar.	5	7863	3·95	9·31	3	6372	4·02	8·83
Apr.	5	7545	3·45	9·12	2	8687	4·02	9·36
May	9	7783	3·57	9·14	6	8364	3·57	9·07
June	4	7890	3·58	8·97	5	10417	3·60	8·94
July	6	6718	3·75	9·16	6	7091	3·67	9·06
Aug.	5	7661	3·54	9·14	3	7795	3·83	9·18
Sept.	9	7157	3·90	9·23	5	7274	3·93	9·39
Oct.	4	6526	3·67	9·15	5	7385	3·76	9·17
Nov.	13	8776	3·67	9·16	6	7998	3·64	9·07
Dec.	7	7642	3·56	9·13	2	9135	3·68	9·06

The same groups of figures were used to determine the degree of relationship between the lactation yields and the corresponding milk-fat and solids-not-fat percentages. Owing to the need for larger numbers in this part of the study on variations due to age, monthly changes in the relationships were examined in the groups for first and second calvers only. Correlation and partial correlation coefficients were calculated for each age group. For the first and second calvers the coefficients for the whole groups were obtained as averages of the monthly coefficients using the z transformation factors to facilitate the process (11). The results of the calculations for each month of the year are presented in Table 5 and the total and partial correlation coefficients for all months in Table 4. A correlation coefficient was calculated between lactation yield and lactation butter-fat percentage for all the first calvers that had entered the herd before the year 1935. The same method for selection and modification of the data was employed as that used for the other set of figures. The result is included in Table 4.

Table 4. *Correlation coefficients and partial correlation coefficients for different age groups of cows*

(Lactation milk yield (lb.) = a. Lactation milk-fat percentage = b. Lactation solids-not-fat = c.)

Lactations	Degrees of freedom	r_{ab}	r_{ac}	r_{bc}	Degrees of freedom	$r_{ab.c}$	$r_{ac.b}$	$r_{bc.a}$
First	104	+0·037	+0·019*	+0·383**	103	+0·032	+0·005	+0·382**
Second	61	−0·162	−0·052	+0·423**	60	−0·155	+0·019	+0·420**
Third	77	−0·119	+0·098	+0·222*	76	−0·145	+0·128	+0·236*
Fourth	50	−0·004	+0·112	+0·014	49	−0·003	+0·112	+0·014
Fifth	29	−0·212	+0·273	+0·568**	28	−0·072	+0·481	+0·666**
Sixth	14	+0·436	+0·121	+0·356	13	+0·424	−0·043	−0·340
Seventh	9	−0·262	−0·035	+0·601	8	−0·302	+0·158	+0·638*

Correlation coefficient for lactation milk yield and lactation milk-fat percentage, calculated from records accumulated before January 1935. First lactation only.
$r_{ab} = -0{\cdot}226^{**}$; degrees of freedom 119.
* Significant when $P = 0{\cdot}05$. ** Significant when $P = 0{\cdot}01$.

RESULTS

The means of age groups presented in Table 2 follow a regular pattern; the milk yields increase in value with the lactation age of the cows. At first the increase is rapid, but after the third lactation the rate of increase is much slower and a maximum is reached in the sixth lactation. There is no regular change in the values of the coefficients of variation, and it is probable that the differences that do occur are due to chance. The means of milk-fat and solids-not-fat percentages show a regular fall as the lactation age of the cow increases, and the accompanying figures estimating the coefficients of variation show a trend in the opposite direction up to the fifth lactation.

The lactation averages grouped according to the month of calving that are given in Table 3, do not exhibit any seasonal trends. With three exceptions the large number of correlation and partial correlation coefficients given in Tables 4 and 5, measuring the degree of relationship between lactation milk yield and both lactation milk-fat and lactation solids-not-fat percentages, fail to reach statistical significance. This is true for the estimates of the different age groups and for the groups of first and second lactations that were subdivided according to the month of calving. The most notable exception to the rule that these coefficients lack statistical significance is the coefficient for lactations

Table 5. *Seasonal variations in correlation coefficients*

(Lactation milk yield (lb.) = *a*. Lactation milk-fat percentage = *b*. Lactation solids-not-fat percentage = *c*.)

Month of calving	First lactations				Second lactations			
	Degrees of freedom	r_{ab}	r_{ac}	r_{bc}	Degrees of freedom	r_{ab}	r_{ac}	r_{bc}
Jan.	4	+0·118	+0·809	−0·281	6	−0·151	+0·473	+0·690
Feb.	7	−0·171	−0·373	+0·323	4	−0·770	−0·837*	+0·806
Mar.	17	+0·442	+0·147	+0·491*	7	+0·003	+0·512	+0·705*
Apr.	5	+0·596	+0·020	+0·289	6	+0·214	−0·433	+0·351
May	9	+0·267	+0·295	+0·383	6	−0·324	+0·031	+0·004
June	5	−0·156	−0·352	+0·939*	3	−0·014	+0·213	+0·173
July	0	—	—	—	1	−0·592	−0·494	+0·895
Aug.	12	−0·047	−0·137	+0·496	9	−0·052	+0·162	+0·595
Sept.	17	−0·364	−0·116	+0·632*	10	−0·015	+0·037	+0·801*
Oct.	12	−0·581*	+0·137	−0·203	7	−0·520	−0·167	+0·705
Nov.	16	+0·310	+0·042	+0·125	7	−0·160	−0·159	+0·371
Dec.	11	+0·176	+0·098	+0·357	7	−0·087	−0·159	−0·238

* Significant when $P = 0{\cdot}05$.

completed before 1935, measuring the degree of relationship between lactation milk yield and lactation milk-fat percentage. In this case the coefficient reached a fairly high level of statistical significance, though its magnitude is low.

The other two exceptions, first lactations for October and second lactations for February, the coefficients for lactation milk yield and milk-fat percentage in the first case and milk-yield and solids-not-fat percentage in the second case, reached a value comparable with statistical significance, but the large number of coefficients calculated greatly increased the probability that individual coefficients would reach high values by chance, and it is considered that this is the explanation of the high values found here; no significance has been attached to them.

The correlation and partial correlation coefficients measuring the degree of relationship between lactation milk-fat and solids-not-fat percentages are not of a very uniform magnitude but, nevertheless, the relationship must be given statistical significance. There is no evidence of any seasonal trend in the estimates, but there is a very slight indication of an increase in value with the higher age groups of cows.

DISCUSSION

Coefficients of variation for different lactation age groups

The results show unequivocally that in these data the records of first lactations should be studied in preference to those of any other age grouping of lactations; not only are the coefficients of variation for milk yield milk-fat percentage and solids-not-fat percentage least for this age group, but the greater number of records increases the advantage when standard errors of means are calculated. Although the coefficient of variation of milk yields for the first lactations is at the minimum value for the series, there is little evidence of any gradient with increasing age among the other coefficients to support the claim made by Roberts (12) that the yield of cows over 5 years old was more variable than that of younger animals, although the values of the coefficients accord well with the values 21·94% for milk yield and 7·8% for butter-fat percentage found by Johansson & Hansson (13), and fairly well for those of Gains & Davidson (14), also for bulked age groups. The variability of both butter-fat and solids-not-fat percentages appears to increase with the age groups

studied, at least with the younger and numerically larger groups. These trends support the low figures for the first age group and strengthen the case for a study of first lactations; the claim of Roberts (12) that there is no trend with age of the variability of butter-fat percentages is not supported by the evidence of this study.

The evidence that first lactations appear most suitable for survey studies should not be lightly extended to the planning of specific experiments; in such cases it is often desirable to maximize the response to an experimental treatment; older cows are known in some instances (15) to respond with greater freedom so would be preferable as experimental animals.

The variation in lactation milk yield due to the age of the cow

The actual levels of the mean values are of little interest save for the measure they afford of the capabilities of the animals under investigation. The relation of the mean values to each other is of more interest. Owing to the method of calculation the trend with age of the mean lactation yields does not provide an unbiased estimate of the true curvilinear regression, due to the probability that the means of the older age groups, in relation to those of the younger groups, are exaggerated in value; this is due to the removal by culling after one or two lactations of those animals that showed least promise of developing into mature cows of acceptable standard. If these cows had continued in the herd their below average yields would have depressed the average yield of those better cows that had been kept in the herd as long as possible. The bias should make no difference to the time of peak yields, and in fact the maximum occurs at the sixth lactation which corresponds with the conclusion drawn from figures from which the bias has been eliminated (16).

The variation in milk composition due to the age of the cow

The same criticism of bias in the regression of milk yield on age can be made for the two comparable regressions involving butter-fat and solids-not-fat percentages and age; however, in these cases the bias is probably somewhat less. Owing to the need to keep up the numbers of a self-contained herd, only a few animals have been culled because of the poor milk-fat content of their milk and practically none because the solids-not-fat of their milk was not up to standard.

Some of the earlier workers (17, 13, 18), reported that the trends of lactational butter-fat and solids-not-fat percentage with age were not linear, but that second lactations produced the richest milk. In this work the negative trends appear substantially linear and first lactations undoubtedly produced the highest mean values.

The rate of the decline reported here, for the solids-not-fat mean figures, is somewhat greater than those usually reported (0·36 %) during the first seven lactations compared with (−0·21 %) (19) and (−0·23 %) (17) over the same range. This could in part be due to the difference in the method employed by the earlier workers and that used here, to calculate the solids-not-fat percentages with and without reference to the milk-fat content of the milk. The greatest difference that this could account for in the present case is 0·04 %; as the difference to be accounted for amounts to about 0·15 % there is a considerable fraction of the difference for which there is no explanation.

The fact of the difference in the rate of the decline is the more surprising when it is recalled that records of milk quality have been available for study when decisions as to culling have been made. It would be expected that this would lead to some selection in

the older cows for milk of above average quality. It must be remembered, however, that the population of cows studied by White & Drakeley (17) was also selected for high milk yield and high quality, so the difference between the rate found by them and that found here may be due to greater selection among their cows. Alternatively, the difference may be due simply to chance or to the effect of the experiments carried out with the cows studied here. If the greater rate of fall found here is a true estimate and is typical of many herds, then other things being equal, any change in the average age of the milking cows in this country has been attended by changes in the quality of the nation's milk far larger than has been supposed.

Variations in lactation records due to seasonal calving

The monthly mean figures of Table 3 do not reveal any consistent advantage in lactation records from arranging calvings to fall at any particular season of the year. This is an advantage from the survey point of view, for it means that no corrections need be made for season variations in any of the records; further, if desired, a selection of records taken from any period of the year may be used without obtaining a biased result.

The absence of any seasonal variations does not agree with the usual experience that October to February calvings produce the greatest lactation milk yields (20). The reason put forward is that cows calving at that time are at the optimum stage in lactation to benefit from the spring flush of grass; in consequence the period of peak production of milk is greatly extended. The existence of seasonal variations in milk yield has been attributed to bad management, possibly bad feeding practice during the summer months. It follows that the absence of seasonal variation may be due to good management. Alternatively, it may in this case be due to the inadequacy of the numbers of cows studied, or to the effect of experiments, usually taking place in the winter months and in most cases involve many of the newly calved animals in full milk, which are just those that in other non-experimental herds would gain the maximum advantage from calving at that season.

Variations in correlation coefficients due to age and to mastitis infection

The values of the correlation coefficients presented in Table 4, are, with the exception discussed later, very similar to those found by other workers. On theoretical grounds it can be argued that outbreaks of mastitis can bias the value of correlation coefficients. Unfortunately, the relative effects of the disease on yield, milk-fat and solids-not-fat percentages are not known (but see Crossman, Dodd, Lee & Neave (21)), although the absolute effect is probably small (22); however, there can be no doubt that they are each depressed below their average values; mathematically this implies a shift of the variates towards the lower limits of their range, if such variates were treated with those of unaffected cows the result would be a bias in the direction of a positive correlation. Murphy (23, 24) has shown that infections of the mastitis caused by *Streptococcus agalactiae* are more frequent with old cows than young. Because of this the sign of a correlation should tend to change with the advancing age of the group of cows studied and progressively gain in positive value. It follows that correlations calculated from data for first lactations would be least biased and therefore best for survey studies. In spite of the attempt in this work to eliminate records of diseased cows, there is some rather weak

evidence that the value of the correlation between milk-fat and solids-not-fat percentages has increased with the age of the group of cows studied; it may be due to an increased incidence of mastitis.

Variations due to herd management

In this investigation the change in value from $r = -0{\cdot}226$ for the correlation coefficient of lactation milk-yield and lactation milk-fat percentage calculated from the set of figures accumulated before 1935 to the negligible value for the main set of figures, is a feature that requires discussion. The most probable cause of such a change would appear to be a drive to improve milk quality; a herd policy involving breeding from the best families of cows and culling the worst would do this. In fact, a considerable reduction in the number of families of cows in the herd has taken place; considerations of milk yield and milk quality have played their part in determining this, so it can be fairly said that relative to the cows present in the herd before 1935, the cows more recently in the herd represent a selected population. The effect of calculating correlation coefficients from selected populations of cows has been discussed by Gains & Davidson (14); by the selective standards of the American Advanced registry of dairy cows, it was found that increased selection increased the value of the negative correlation. Different standards, such as those that have been applied to the Institute herd, could have reversed this tendency and resulted in the loss of any correlation.

The fact that changes can be brought about in the value of this correlation is of considerable interest; it shows that a farmer need not accept as inevitable the regular association of high yield with low milk quality, but that by suitable methods a population of dairy cows could be brought together which would exhibit the ideal characteristics of above average yield and above average milk quality. It may be that it is only the association of milk yield and milk quality that can be so altered, for with an unselected population the values of the correlations are always very low, so the association is naturally rather a weak one. In the case of milk quality, that is the association of lactation milk-fat and solids-not-fat percentages, the value of the correlation appears to be greater; in consequence the degree of selection necessary materially to change its value, would have to be much more rigorous.

When correlation coefficients are considered in relation to breeding policies it is very necessary to remember that neither the relative heritability nor the relative dependence on managerial or feeding practice are known for the three lactational records, milk yield, milk-fat and solids-not-fat percentages, it follows that should a farmer select cows on the basis of only one of these measurements of performance he cannot place any estimate either in degree or in time on the effect he will produce on the other measurements.

Perhaps the most popular breeding policy practised by farmers in this country is selection for high milk yield without regard for the quality of the high yielding animal's milk; the method of payment for milk mainly on the basis of gallonage only must encourage such a policy. The breeding policy referred to is usually applied in the first instance to an unselected population of cows, within which the natural negative correlation exists. The selection of the highest yielding cows from such a population must inevitably tend also to choose those cows whose milk is of poor quality. The decline in much of this country's milk (3) is justly ascribed in part to this form of breeding policy (2, 25, 22).

Perhaps one of the reasons why the long-term decline in milk quality is so difficult to

reverse is that selection of cows for high-quality milk, from a population already selected by this breeding policy, is handicapped by the loss of so many cows whose genetic make-up offers the greatest opportunities for milk-fat improvement.

More progressive farmers base their breeding plans on records of milk yield and its milk-fat content; in this case, according to the standards used, the value of the correlation between these two measures can be expected to change and the levels of both milk yield and its milk-fat content to rise. The effect on the solids-not-fat content of the milk cannot be estimated with any accuracy; its level is very unlikely to fall owing to the existence of the positive correlation it has with the butter-fat content of the milk, but no rapid or profitable increase can be relied on. It must be remembered that a correlation coefficient of only 0·4 allows for a considerable minority of pairs of values with little association, so that in a herd of cows, individual members of it may be expected to produce milk of above average butter-fat content and below average solids-not-fat content and vice versa. The only dependable way of improving the solids-not-fat content of the milk of individual members of a herd is through the operation of a breeding policy to select cows directly for the high value of the solids-not-fat content of their milk.

SUMMARY

1. The coefficient of variation of lactation milk yield was approximately 25% for all age groups. That for lactation milk-fat percentage approximately 10% and that for lactation solids-not-fat percentage only 3%. The last two coefficients tended to increase with the age of the cow.

2. Lactation milk yield was least with first calvers and rose to a maximum for sixth calvers. Milk quality was highest with first calvers.

3. No tendency to vary with the season of calving could be found for any lactation record of any age group.

4. During the first years of the herd's existence there was a small negative correlation between lactation milk yield and lactation milk-fat percentage. This has disappeared in more recent years.

5. There was no correlation between lactation milk yield and lactation solids-not-fat percentage.

6. There was a small ($r = +0{\cdot}40$ approximately) positive correlation between lactation milk-fat percentage and lactation solids-not-fat percentage.

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466. STUDIES ON VARIATIONS IN THE SOLIDS-NOT-FAT CONTENT OF MILK

II. VARIATION DUE TO STAGE IN LACTATION

By G. L. BAILEY
National Institute for Research in Dairying, University of Reading

(With 3 Figures)

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INTRODUCTION

Variation due to stage in lactation is inherent in all within-lactation studies on milk quality and milk yield.

So far as is known no stage-in-lactation curves for solids-not-fat percentages have yet been prepared from figures obtained by the highly accurate gravimetric method of estimation. The work described in this part is devoted to the production of such curves, and the results are discussed in the light of the use to which the curves may be put, so that the accuracy of further survey studies may be increased.

Early work on this subject was done by Bartlett (1), who used figures of solids-not-fat determined by the lactometer method. Discussing his results he remarked on the possible errors in the shape of his curves that might be due to this method and, in addition, he stressed the need for further work when more reliable figures were available. His study was also carried out on data from the Institute herd. Section (1) of this part may therefore be regarded as a continuation of his work.

(1) THE STANDARD CURVE

Statistical method

The data available were arranged so that the records of the monthly estimations of solids-not-fat percentages were divided into three groups. These groups consisted of records from first lactations, second lactations and older lactations. Each group was subdivided so that pregnant and barren cows were treated separately. Very short and very long lactations were omitted or modified to eliminate unhealthy animals by the method used

by Bailey (2). By this method each animal, contributing records to the study, supplied a series of between six and ten figures, one figure for each month of lactation. Every animal had complete records for the first 6 months after calving but, according to the length of the lactation, some had records for a further 4 months. Approximately 80% of the cows had records for the full 10 months.

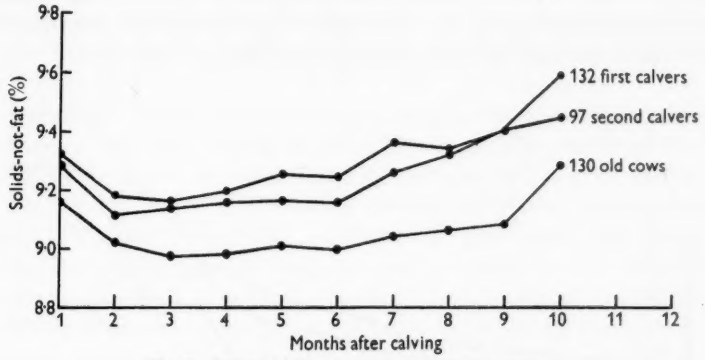

Fig. 1. Stage-in-lactation curves, pregnant cows.

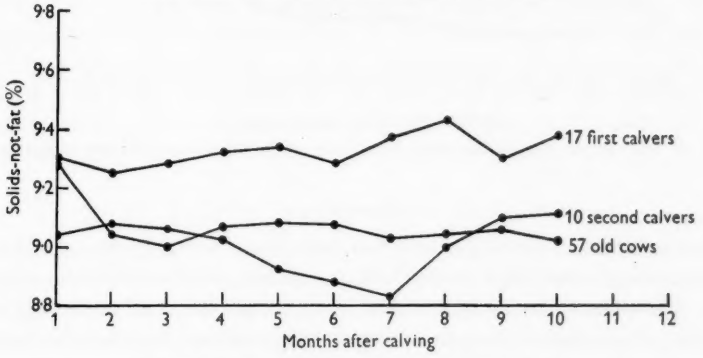

Fig. 2. Stage-in-lactation curves, barren cows.

The very early stages of the lactation curves could not be examined, for the routine tests were not carried out for any cow that had not been in milk 14 days; in consequence the range of the interval between calving and the first test was between 14 and 45 days, and an average time interval of 1 month can be assumed as a good estimate.

From the data available stage-in-lactation curves from the end of the first month to the end of the tenth month of lactation were prepared. Curves for the pregnant animals of each group are given on Fig. 1 and for the barren animals on Fig. 2. The influence of the date of conception on the shape of the lactation curves was examined by drawing curves (Fig. 3), with only those animals successfully served during the first 161 days of lactation being included.

Results

The two sets of stage-in-lactation curves drawn on Figs. 1 and 3 together can be regarded as standard. Fig. 1 gives the best impression of the early and middle stages of lactation. The solids-not-fat content of milk is relatively high 1 month after calving, but falls rapidly

and reaches a minimum level during the second and third months of lactation; thereafter there is a slow rise in the level until about the sixth month of lactation. At this stage the question of pregnancy begins to influence the trend, and Fig. 3 shows that about 2 months after successful service, which time commonly occurs in the sixth month of lactation, the rate at which the rise takes place becomes much greater. The gain from the second to the sixth month is for first calvers no more than 0·06%, but in the 4 months from 2 months after conception the gain is 0·42%. Fig. 2 shows that in the case of barren cows this considerable rise in the level of solids-not-fat in milk does not take place.

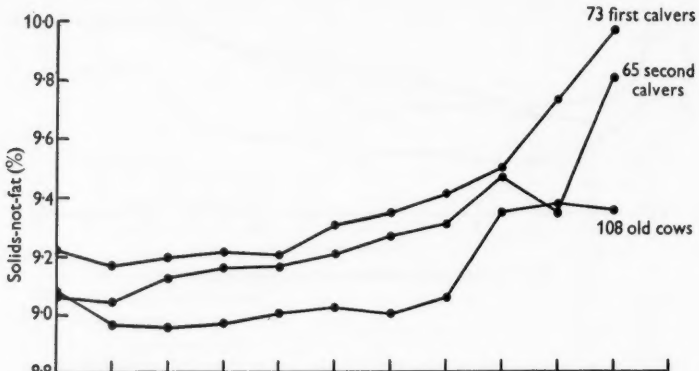

Fig. 3. Stage-in-lactation curves, cows successfully served before the 160th day of lactation.

Discussion

The stage-in-lactation curves prepared here are of most value for the information they provide regarding the best time to start and stop making serial observations of solids-not-fat levels, the series to be as free as possible from the effect of advancing lactation. Furthermore, if the series of observations has to be so extended that the effect is inevitably of some importance, correction factors can be calculated from the curves, so that the variation due to the effect can be kept within small limits.

The fall in the curves that occurs during the first 6 weeks of lactation is so pronounced that, owing to the difficulty of accurately measuring the slope for such a short period, serial observations should not begin before the middle of the second month. From the middle of the second month to the middle of the sixth month there is a smooth section of the curve that shows little change in the level of the solids-not-fat figures; this appears to be the best period of time for making a series of observations, for the effect of advancing lactation is at a minimum, and, if necessary, correction factors can be estimated and applied to reduce the effect to still smaller limits. It is noteworthy that during this time the curves drawn from pregnant and barren animals are not very different, so that both groups of animals can be treated together without much danger of the groups behaving differently.

After the sixth month of lactation the rise in the curves for the pregnant animals becomes much more rapid, and the curve appears rather less smooth so the use of correction factors to eliminate the effect of the rise must be less reliable. For these reasons the

period after the sixth month must be regarded as a less favourable time for making serial observations of solids-not-fat. A further consideration arises at this time, for the rapidly widening departure of the curve for the pregnant cows from that of the barren cow precludes the use of both groups of animals in one class.

In a planned experiment where the animals employed can be selected so that the effects of advancing lactation can be experienced equally by treated and control animals, the conclusions drawn from the shape of the curves can be applied to the selection of the experimental animals, for they point out the limits beyond which cows should not differ in stages in lactation.

The outstanding point is to ensure that experimental cows between 6 weeks and 6 months in milk are not matched with animals that have been in milk for periods outside this range. For the study of solids-not-fat figures already existing, such as those for the National Institute for Research in Dairying herd, the same stringent selection is necessary.

The forms of the curves presented here correspond quite closely with those predicted by Bartlett(1) for curves prepared from gravimetric estimations of solids-not-fat percentages, although the rate of increase in levels is perhaps even larger than expected.

Consideration of the curves for animals pregnant within 160 days of parturition leaves no doubt that pregnancy is the major factor in determining both the fact and the time of the onset of the increased rate of the rise; in most cases this starts 2 months after the date of conception. This is sooner than reported by Bartlett(1), and the difference is almost certainly due to the different methods used to estimate the solids-not-fat figures.

The difference in levels of the curves for the animals of different age groups is in agreement with the results of Part I. The possibility that the form of the curves for different age groups might differ, has been considered in § (2) of this Part.

(2) VARIATION IN THE GRADIENT OF THE CURVE

Statistical method

Data from all the Shorthorn cows in the N.I.R.D. herd that completed lactations of at least 280 days during the years 1935–49, both years inclusive, were gathered and grouped into lactation age groups; data from pregnant and barren cows were kept separately. The data concerning the solids-not-fat content of the milk were obtained from the records of the routine monthly tests, and for each cow the series of eight figures from this source, covering the second to ninth months of lactation, was the part of the available data actually used. The mean value (x_1) of each of these series was calculated, and in addition the mean gradient (y) of the stage-in-lactation curve for each series was obtained by the calculation of a linear regression coefficient of solids-not-fat percentage on months of lactation. The omission from the series of the figures of the records of the tests made after only 1 month of lactation and again all those after the ninth, was dictated by the use of a linear regression coefficient to describe what is in fact, curvilinear. Over the range actually used, the error arising from this assumption is small; the effect of the error is greatest at the range limits, at the beginning of the period the gradient is overestimated and at the end underestimated. Extrapolation to the first month of lactation is not justified, but if carried beyond the ninth month of lactation, no very serious error results.

It was shown in § (1) that the gradient of the stage-in-lactation curve becomes more marked about 2 months after the date of conception, so that the mean gradient (y) of the curve varies with the calving interval (x_2). In order to eliminate variation in the mean

gradient due to this source the calving interval measured in days (x_2) was tabulated with the other variables. The data were subdivided within age groups according to the mean value (x_1). The ranges of the groupings, together with the means of each division's three variables, are given in Table 1; details of the first three age groups only are given, owing to the small numbers of cows contributiong to the older age groups.

Table 1. *Mean gradients of stage-in-lactation curves*

Age	Range of groups of mean values (%)	n	Mean (x_1) S.N.F. %	Mean calving interval (x_2) (days)	Mean gradient (y) % per month
First calvers	<8·99	8	8·91	451	+0·0461
	9·00–9·19	26	9·11	428	+0·0247
	9·20–9·39	52	9·29	423	+0·0560
	9·40–9·59	38	9·47	424	+0·0698
	>9·60	8	9·70	410	+0·0917
Second calvers	<8·99	16	8·87	416	+0·0074
	9·00–9·19	30	9·11	419	+0·0417
	9·20–9·39	27	9·29	423	+0·0408
	9·40–9·59	15	9·47	415	+0·0696
	>9·60	5	9·68	387	+0·0726
Third calvers	<8·79	5	8·66	440	−0·0178
	8·80–8·99	12	8·91	400	+0·0310
	9·00–9·19	17	9·09	449	+0·0541
	9·20–9·39	12	9·29	390	+0·0588
	9·40–9·59	8	9·48	408	+0·0983
	>9·60	4	9·72	425	+0·1030

The rate at which the mean gradient (y) changed with changes in the mean value (x_1) with the effect of changes in the mean calving interval (x_2) eliminated $b_{yx_1 \cdot x_2}$, was calculated for each of the first four age groups and for barren cows. The results of these calculations, together with the means of the three variables for the age group totals, are given in Table 2.

Table 2. *Changes in the mean gradient of different stage in lactation curves*

Age group	n	Mean S.N.F. (x_1) %	Mean S.N.F. (x_1) S.D.	Mean calving interval (x_2) Days	Mean calving interval (x_2) S.D.	Mean gradient (y) % per month	Mean gradient (y) S.D.	$b_{yx_1 \cdot x_2}$ and its standard error
First calvers	132	9·31	0·193	427	73·9	0·0551	0·0545	0·0485±0·048
Second calvers	93	9·21	0·229	417	61·4	0·0410	0·0566	0·0574±0·026
Third calvers	58	9·16	0·285	421	71·7	0·0551	0·0650	0·1219±0·025
Fourth calvers	34	9·12	0·263	417	62·6	0·0387	0·0700	0·1904±0·136
Fifth calvers	17	9·05	0·572	416	13·2	0·0121	0·0456	—
Sixth calvers	11	8·87	0·242	433	65·5	−0·0114	0·0866	—
Barren cows Average age 3·05 lactations	58	9·09	0·314			0·0056	0·0625	0·1113±0·022

Results

It has been shown quite clearly that if a cow produces milk with a relatively high solids-not-fat content, the advantage over a cow that produces milk of only average or low quality will be greatest at the end of lactation and least at the beginning, even though the quality of the milk from the average cow will become higher as the period of lactation increases. To illustrate this, figures taken from Table 1 show that if a cow in her third

lactation, 5½ months after calving, produces milk with a solids-not-fat content of 8·91%, this might be expected to improve by about 0·0310% for each month of the second to ninth month of lactation, while a cow of the same age producing milk at the same stage in lactation of 9·48% solids-not-fat, would be expected to improve by 0·0983% for each month. Comparable figures may be calculated from the data of Table 2; in the case of the first cow the milk would be expected to improve by 0·0253% per month and that of the second cow by 0·0947% per month. In view of the errors that must be attached to these estimates and the small difference between comparable estimates, it is reasonable to think that the estimates obtained in the two different ways are substantially the same for each cow. If this is true, the use of the regression gradient on months ($b_{yx_1 \cdot x_2}$) is justified within its appropriate age group over the whole range of the mean solids-not-fat percentage variates (x_1); in other words the regression is linear.

The results given in Table 2 show considerable differences between the age group means. The fall with advancing age in the mean S.N.F. % (x_1) and the concomitant rise in the standard deviations is to be expected and conforms with earlier observations. There is no apparent trend in the series of mean calving intervals (x_2). The downward trend in the series of mean gradients (y) is not very smooth, yet it seems probable that the irregularities are due to chance. The considerable value of the standard deviations of these estimates of mean gradients is not so surprising when it is borne in mind that the mean gradients represent a mixed population in that the individual variates vary considerably with the highly variable individual solids-not-fat percentages (x_1).

The rates of changes in the gradients (y) with the changes in the mean values (x_1) show a striking trend with the increase in the age groups. To illustrate this, if a fourth calver has a mean S.N.F. % in her milk of 9·30 the improvement from month to month might be expected to be 0·0735%, while a first calver with the same mean value, should improve only 0·0550% per month. It must be borne in mind when making these estimates that very considerable errors attach to them, indeed the two coefficients used here, do not, by themselves, reach statistical significance; however, they are supported by the two statistically significant coefficients for second and third calvers, so their use appears to have some justification.

The results for the barren cows may be compared directly with those for the third calvers, as the average age of the two groups is very nearly the same. The milk of a barren cow in her third lactation with a mean S.N.F. % of 9·30 would improve by only 0·0285% per month, while the milk of a pregnant third calver would improve by 0·0725% per month. The very slight gradient of the S.N.F. % stage-in-lactation curve for barren cows has been noted already in the first part of this section. It is noteworthy, however, that the rate of change in the gradient (y) with changes in the mean value (x_1) is substantially the same as for pregnant cows of comparable age.

DISCUSSION

The mathematical relationships found in this study should find practical expression in the field of animal experimentation. The findings will enable a closer control to be exercised over the variations in the S.N.F. % in cow's milk and so lead to economy in the use of experimental animals and in the easier detection of small treatment differences.

It has been shown in humans by Goldberger & Frank (3) that the production of oestrogens by the pregnant female greatly increases as pregnancy advances. Folley,

Watson & Bottomley (4) have shown in dairy cows that the administration of oestrogens to the lactating cow produces a marked rise in the solids-not-fat fraction of her milk. These facts suggest the possibility of a correlation between the level of blood oestrogens and the solids-not-fat content of milk. The results found here could be of great value should research on these lines be undertaken; they would enable an estimate to be made of the most suitable cows to study so that comparable results would be obtained.

SUMMARY

Curves are presented showing the trends in the solids-not-fat fractions of milk during the course of normal lactation periods.

The trends may vary with pregnancy, age of the cow, and the mean level of the solids-not-fat.

My thanks are due to Dr S. Bartlett for his interest and helpful advice, to Mr J. Sears for help in marshalling the data; also to Mr W. A. Wagstaff, who carried out most of the milk analyses, and to Mr B. Ridler, who kept the farm records necessary for this work.

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467. STUDIES ON VARIATIONS IN THE SOLIDS-NOT-FAT CONTENT OF MILK

III. SEASONAL VARIATIONS

By G. L. BAILEY
National Institute for Research in Dairying, University of Reading

(With 3 Figures)

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INTRODUCTION

Realization of the importance of the fall in the solids-not-fat content of late winter milk (1, 2) has led to an interest in seasonal variations as a whole, and a desire to understand more about the factors bringing about the changes. The records available at the National Institute for Research in Dairying, which were collected under rigorously controlled conditions, provide material for a thorough study of the subject.

STATISTICAL METHOD

The reliability of any estimate of seasonal variations in milk quality depends, of course, on the accuracy of the original data. In addition it depends on two factors. The first of these is the number of cows whose milk is studied, the second is the extent to which the cows whose milk is examined in each month can be regarded as random samples from the same population. In most investigations the milk of a large number of cows is studied, and this is presumed to preclude bias in the estimate of seasonal variation.

For the investigation reported here this course was not possible owing to the comparatively small number of the cows; consequently a close control has been kept on the character of the cows and estimates should therefore be unbiased even if sampling variations are somewhat large.

The seasonal variation of milk from cows in their first lactations has been studied separately from that of other age groups. This has been done because first calvers constitute the largest single age group. The others, if treated separately, would not be sufficiently large to provide estimates for each month of the year. The advantage gained by treating one age group by itself is that, when a seasonal curve is computed from data of a whole survey period, there cannot be two or more figures from any one cow contributing to any monthly mean value, so that the individuality of a particular cow cannot bias the results.

The seasonal variations have been studied for each month of the year from 1935 to 1946. The effects of advancing lactation have been shown by Bailey (3) to be least during

the second to sixth months, consequently in this study estimates of solids-not-fat percentages made during this time only were used; correction factors estimated from the lactation curves drawn by Bailey (3) were applied to the figures to reduce the effects of lactation to still finer limits. The factors used are given in Table 1. No distinction was made between pregnant and barren animals.

Table 1. *Table of correction factors obtained from the stage-in-lactation curves drawn by Bailey (3); the curves were slightly smoothed to obtain idealized factors*

	Months after calving				
Age of cow	2	3	4	5	6
First lactation (%)	−0·02	0	−0·03	−0·05	−0·06
Second lactation (%)	0	−0·01	−0·02	−0·03	−0·03
Old cows (%)	−0·02	+0·02	0	−0·01	−0·01

Table 2. *Table of monthly mean differences between pairs of months*

Pair of months	First calvers Monthly mean difference (%)	Old cows Monthly mean difference (%)
Jan.–Feb.	+0·033±0·022	−0·017
Feb.–Mar.	−0·027±0·042	−0·027
Mar.–Apr.	−0·023±0·033	+0·015
Apr.–May	+0·173±0·032**	+0·139
May–June	−0·104±0·040*	−0·107
June–July	−0·098±0·040*	−0·189
July–Aug.	+0·050±0·034	−0·006
Aug.–Sept.	+0·105±0·024**	+0·126
Sept.–Oct.	+0·042±0·062	−0·052
Oct.–Nov.	−0·075±0·079	+0·013
Nov.–Dec.	+0·011±0·070	+0·029
Dec.–Jan.	+0·044±0·064	+0·023

* Significant when $P = 0{\cdot}05$. ** Significant when $P = 0{\cdot}01$.

The selected and adjusted figures were tabulated according to the year and the month in which the tests were made; comparisons were then made between pairs of months using figures from animals whose milk had been tested in both months. Figures from an average of approximately five cows were available for each comparison. The results of these calculations were plotted as discontinuous curves (Figs. 1, 2). An average curve for the 12 years was also plotted (Fig. 3). The average monthly mean differences obtained by allocating equal weight to monthly figures for each year are shown in Table 2. This includes, with first calvers, an estimate of the error involved in the calculations, thus providing a guide to the significance that can be given to each of the differences.

RESULTS

The discontinuous curves drawn on Figs. 1 and 2 show the way in which the level of solids-not-fat in the milk produced by the two groups of cows studied varied from month to month for each of the 12 years of the survey. Except at certain seasons of the year there is little similarity between the behaviour in the same year of the two groups of cows. These both experienced the same seasonal conditions, so when the trends in the level of solids-not-fat of milk from the two groups of cows were different, the influence of season on the level of solids-not-fat must have been too small to have had any measurable effects. Where there are gaps in the curves, the number of cows contributing figures to the survey was too small to make an estimate of the changes in the levels for the particular months.

The average seasonal trends in the solids-not-fat of the milk from the two groups of cows are shown in Fig. 3 and Table 2. These point out the times of the year when changes

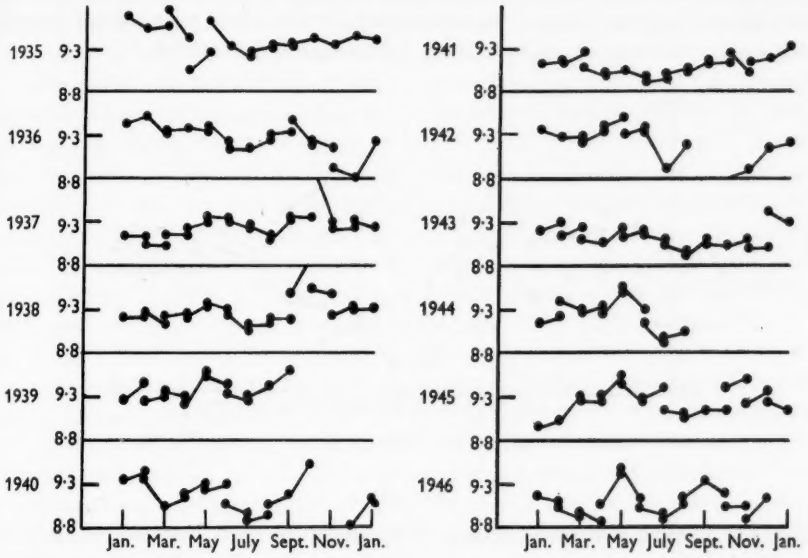

Fig. 1. Seasonal variations in the solids-not-fat of milk from first calvers.

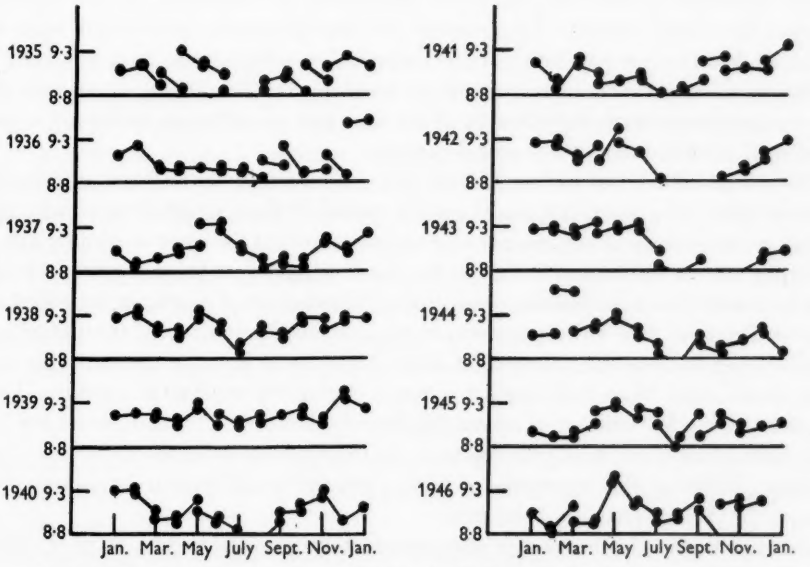

Fig. 2. Seasonal variations in the solids-not-fat of milk from second calvers and old cows.

in the level of solids-not-fat occurred regularly; such changes occurred from April to July and again from August to September. At other times of the year the changes in the levels

are only small and appear to have taken place at random. From April to May the level rose sharply; for first calvers it amounted to 0·173%. This was followed by a fall from May to July amounting to 0·202%, which was so large that in consequence the level reached during July and August was the lowest for the whole year. From August to September the level rose again, by 0·105% in the case of first calvers.

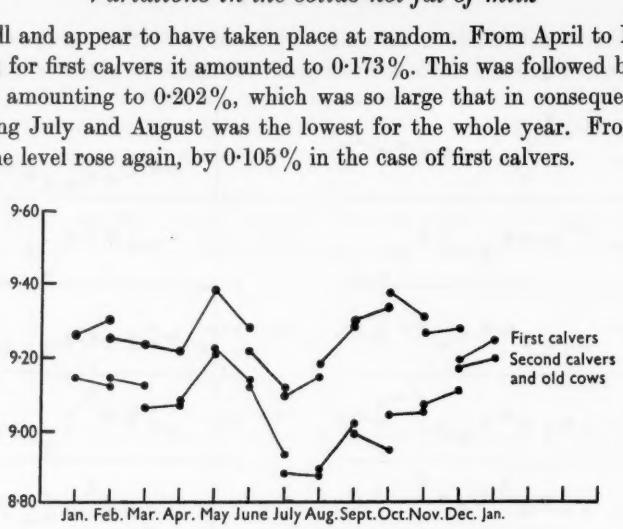

Fig. 3. Average seasonal variations for the 12 years 1935–46.

DISCUSSION

Statistically significant differences have been shown to exist between monthly mean levels; these differences are not due to changes in the characters of the cows studied and therefore must depend on seasonal feeding or managerial practices.

The statistical method employed has maximized the reliability of the differences between the monthly means. The actual levels must always be considered in relation to them. Unless the cow population studied remained constant for at least 3 months, the statistical method produced two means for each month. The difference between these represents changes in the individuality of the cows and the difference between the means of pairs of months denote the seasonal changes.

The absence of any regular trend in the figures from December to March is interesting. It shows that the cows of this study are not typical of those reported by Rowland (1, 2). Owing to the absence of any control over the characters of the cows studied by him and the strict control maintained in this study, the possibility that the difference in findings may be due to this cause must be considered. Of the factors of character controlled here, stage in lactation of the cows appears to be the one most likely to cause the decline in the herds studied by Rowland. Seasonal calving might result in many cows arriving at the stage in lactation when milk quality is lowest during the months in question. Unfortunately, no method exists of checking this directly; however, Provan (4) found low levels from both autumn and spring calving herds, so this explanation of the difference appears unlikely. Differences in managerial or feeding practice would seem to be more probable reasons for the differences in behaviour.

Although workers in one country all report the same seasonal trends, between different countries the seasonal trends are not necessarily the same; the common feature is that low levels are found when the weather conditions limit the supply of natural grazing. In this country (see Cranfield (5)), when there is a flush of grass, there is always a high level of solids-not-fat in the milk.

In general, the form of the seasonal curves found here is very similar to that prepared by Richmond (6) and that for the eastern part of the country prepared by Provan (7). These curves vary in level with the phases in the growth of grass. The spring flush of grass, when grazing is known to be of very high quality, occurs in April, usually in the latter half of the month or in the first days of May. This coincides with a marked rise in the percentage of solids-not-fat of the milk. As the grass becomes mature during the following 2 months the quality of the grazing falls off, a trend faithfully followed by the solids-not-fat levels. In July and August, when pasture land is very often dried up and there is little grazing for the cows, the solids-not-fat content of milk appears to reach a minimum. The late summer showers and the autumn dews bring new life to the pasture land and the grass again grows actively; the aftermath provides good grazing, but its quality is seldom as high as that afforded by the spring flush; at this time the solids-not-fat content of milk again rises; the mean difference in levels between August and September is statistically significant.

The duration of the autumn grazing and the type of feeding that follows it are both very variable, due to the different weather conditions from year to year. This may account for the increased variability of the differences between monthly mean levels found at this time of the year. The feeding practice for the remainder of the winter months is much more standardized, largely due to indoor feeding. The low standard errors of the monthly mean differences during January, February and March reflect this uniformity.

In the previous paragraph the importance of grazing quality has been stressed. This quality has been assumed to be related to a high nutrient content unaccompanied by any considerable bulk of non-digestible material. This may either in part, or wholly, be a false assumption. Folley, Watson & Bottomley (8) have shown that oestrogenic hormones, when administered to lactating cows, increase the solids-not-fat content of their milk. Oestrogens have been detected in British pasture plants (9); Legg, Curnow & Simpson (10) have found a seasonal change in the oestrogen content of some pasture plants, oestrogenic activity being associated with the phase of reproductive growth. In the light of these observations it is possible that what has been referred to as pasture quality is, in fact, high oestrogen content of the pasture plants, but the theory is not yet supported by sufficient evidence to be accepted completely. Butenandt (11) and Butenandt & Jakobi (12) have reported a high oestrone content of palm kernel residues, which are commonly included in cattle foods and fed at any time of the year. Oestrogenic activity in other cattle foods has not yet been tested, but the work is obviously of importance; if a wide range of such food is found to contain oestrogens the seasonal nature of their intake by cows must be questioned; until this point is cleared up the connexion of milk with a seasonally high solids-not-fat content and oestrogens cannot be regarded as more than an interesting theory.

SUMMARY

Seasonal changes in the solids-not-fat content of milk occur only from April to May when the level rises, from May to July when the level falls and from August to September when the level rises again.

These changes are thought to be due to changes in feeding practice.

My thanks are due to Dr S. Bartlett for his interest and helpful advice; also to Mr J. Sears, who helped to marshal the data, to Mr W. A. Wagstaff, who carried out most of the milk analyses and to Mr B. Ridler, who kept the farm records necessary for this work.

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468. STUDIES ON VARIATIONS IN THE SOLIDS-NOT-FAT CONTENT OF MILK

IV. LONG-TERM TRENDS

By G. L. BAILEY
National Institute for Research in Dairying, University of Reading

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INTRODUCTION

The nutritional importance of milk in this country's human feeding habits is difficult to exaggerate and much of its importance depends on the high-quality proteins of milk which make up a large part of the total non-fatty solids fraction. It follows that the fall in the average solids-not-fat percentage of milk, measured by Provan & Jenkins (1) and the concomitant decline in the amount of protein present in the nation's milk calculated by Kay (2) at 212,000 cwt. in a single year if the total gallonage of milk is assumed to be constant over the years, is a very serious matter. Vigorous action is necessary to do no more than restore the position, while the ideal must be to improve on the earlier solids-not-fat content of milk. A general knowledge of the factors influencing the quality of milk could, if accompanied by wise action, do a great deal of good, but a considerable dissipation of energy might result. This could be avoided by a more precise knowledge of the causes of the decline. An attempt has been made to provide this by a study reported in this Part. The value of the results must depend on the degree to which the performance of a single experimental herd can be regarded as typical of a general population.

STATISTICAL METHOD

For this survey close attention has been paid to the selection and arrangement of data so that the figures used in succeeding years, are from animals very closely comparable in their breed, age, stage in lactation and tested at the same season of the year. The use of records from Shorthorn first calvers only readily achieved the first two of these aims. To obtain figures from cows at the same stage in lactation, the same method was used as that employed by Bailey (3); as a result each cow provided a series of five figures. To achieve the last aim, to remove the effect of season of the year from the figures, it was determined to examine the long-term trends that had occurred in each of the 12 months of the year.

The modified serial figures of solids-not-fat percentages for the selected cows were tabulated in twelve groups, so that each cow contributed only one figure to each of five consecutive groups. The year of test for each of the figures was recorded against the entries in the groups. Owing to seasonal calving a different number of entries was

made for each year in each group; the total number of entries for each group was also different.

Initially the relationships between the years of the tests and the level of the solids-not-fat percentages were assumed to be linear, and linear regression coefficients were calculated by the method of least squares; where these proved statistically significant, the linearity of the regressions was tested. The results of the calculations are given in Table 1.

RESULTS

The trend in the level of solids-not-fat in milk has been shown to be negative for every month of the year except May, when a small positive trend has been estimated. The trends are statistically significant in the months of January, February, March, April and August. The total fall during the 12 years of the survey can be calculated from the regression coefficients given in Table 1, for there is no evidence that the rate of the fall varied from year to year. In the month of April the total fall is estimated at 0·408 %, for August the total fall is estimated at 0·264 %.

Table 1. *Long-term trends in the value of the solids-not-fat content of cow's milk*

Month	No. of cows	Percentage change per year of survey	Variance ratio deviations of means of years from regression/ deviations within years
Jan.	58	−0·028±0·010**	1·15
Feb.	62	−0·029±0·009**	1·18
Mar.	54	−0·029±0·009**	2·03
Apr.	52	−0·034±0·008**	1·87
May	54	+0·010±0·011	—
June	51	−0·012±0·009	—
July	43	−0·018±0·011	—
Aug.	44	−0·022±0·009*	0·89
Sept.	41	−0·015±0·011	—
Oct.	23	−0·042±0·023	—
Nov.	36	−0·030±0·018	—
Dec.	39	−0·005±0·013	—

* Significant when $P = 0{\cdot}05$. ** Significant when $P = 0{\cdot}01$.

DISCUSSION

The existence of a negative trend in the solids-not-fat content of the milk from the Institute's cows has been established, together with the fact that the rate of the fall varies considerably from month to month. Provan & Jenkins(1), who eliminated seasonal variation from their figures by the calculation of 12-month moving average, found that from 1935 to 1946 the level fell about 0·175 %. To compare this with the rate found here an average rate for the whole year has been calculated by weighting each monthly rate by the reciprocal of its error variance; a fall of about 0·25 % in the 12 years has been estimated. The fact that this estimate is the greater of the two is remarkable when it is remembered that so many of the possible causes of the decline have been eliminated, but actually it is very doubtful if the difference between the two rates estimated is real; there is a considerable error term in the estimate made here. It is not possible to argue from the two rates of decline that the factors eliminated in this study have had no effect at all on the general decline; only that the factors remaining are capable of causing a decline as severe as that generally experienced.

The method and material of the survey limit the field of discussion on the possible causes of the decline. Changes in breed popularity, age of the cows and differences in their stages in lactation can all be ignored; further, within each of the twelve groups, there is no bias due to differences in the season of the test. The remaining possibilities appear to be strain within breed and factors of feeding and of management.

The breeding policy practised for the Institute herd has been discussed already (4); from the evidence it appears very unlikely that changes of the strains of cows in the herd could have brought about a fall in the solids-not-fat levels in a cow's milk. This contention is supported by the existence of variations in the degree of the fall from month to month; the effect of a breeding policy would be expected to be constant throughout the year within very narrow limits. Some aspects of the feeding of the cows have been discussed already by Bailey (3). It is noteworthy that, as in that section, the variability of the results is greatest in the autumn months, probably for the same reason. During the first 4 months of the year, when the difficulty of supplying high quality food has been growing greater during the years in question, the rate of the decline is fairly high and the regression coefficient for each month is statistically significant. During these months the total fall in the 12 years is about 0·35 %, a very considerable and serious change. The slight increase in the value of the coefficients from January to April may indicate the increasing difficulty of providing high quality foods during the 'hungry gap' period, or it may reflect an accumulating effect from the experiments.

In the month of May there is no sign of any trend either up or down, an expected result when it is recalled that there has been no change in the feeding practice at this time, when the cows have always obtained the greater part of their rations from grass.

During the following 3 months the grass deteriorates in quality, but the bulk of grazing often present, commonly leads to an overestimation of its value, with the result that the cows may go short. This may have happened to the Institute herd during the war years and brought about the increase in the value of the coefficients from June to August when the coefficient is again statistically significant and when the tendency to overestimate the grass, if alternative food supplies are not available, must be greatest.

It is not possible to prove that this, in fact, occurred, owing to the absence of precise information on grazing quality. An examination of the records of the foods supplementary to grazing given at this time of the year, indicates that during the war years they consisted of approximately half the weight of concentrates given before 1940, but that roughage foods were given in much larger quantities. The result of this appears to have been that although the change in total nutrients may have been small, the cows had to deal with a much larger bulk of food to obtain the nutrients.

The regression coefficients for the remaining 4 months of the year are very variable. In October and November they are high, but the high estimates of error attached to them and the absence of any trend in their values from month to month does not permit the recognition of the coefficients as indicating any real long-term trend.

Eleven out of the twelve coefficients are negative, so that the smaller coefficients are supported in their significance by the larger, even when the estimate of error attaching to them is too large to assume significance from their own internal evidence. However, in this respect, it must be remembered that owing to the method of analysis one cow contributes figures to 5 consecutive months, so some degree of correlation must exist between

the months due to the individuality of the cows. In consequence, the significance to be attached to the smaller coefficients must not be overestimated.

The effect of systems of management on the solids-not-fat content of milk has not received much attention. There is little difference in the solids-not-fat content of first drawn milk and the strippings (5), so efficiency of milking would have little effect. If any other factors of management have any influence on this fraction of milk the effect is probably very small.

Although the tests of linearity on the regressions were statistically not significant, they cannot be taken as conclusive proof that the changes did not occur more rapidly at one time than at another. The changes that appear are thought to depend primarily on feeding practice, and an examination of this aspect has been deferred.

The form of the regressions must have been affected by the inclusion of figures from experimental animals which would not be typical of the figures of non-experimental animals of the same year. This would increase the variability of the means of years as well as the general error, and, in consequence, small trends from year to year would tend to be masked.

SUMMARY

It has been shown that a statistically significant decline in the level of solids-not-fat in milk from Dairy Shorthorn first calvers occurred in the months of January, February, March, April and August. The period of the survey was the 12 years from 1935 to 1946.

My thanks are due to Dr S. Bartlett for his interest and helpful advice; also to Mr W. A. Wagstaff, who carried out most of the milk analyses, and to Mr B. Ridler, who kept the farm records necessary for this work.

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469. THE EFFECT OF TOCOPHEROL AND CAROTENE SUPPLEMENTS ON THE VITAMIN A POTENCY OF NEW ZEALAND BUTTERFAT

By W. A. McGILLIVRAY

Biochemistry Department, Massey Agricultural College, University of New Zealand, Palmerston North

(With 3 Figures)

In 1947, Barnicoat (1) reported results of an investigation carried out during the 1935–6 season into the vitamin A and carotene content of New Zealand butterfat. These results explored, as he pointed out, a somewhat novel field in that they related to the butterfat from animals fed outdoors on pasture throughout the year, the complicating effects due to stall feeding being eliminated. Under these conditions a uniformly high vitamin A potency might have been expected, but Barnicoat found marked seasonal fluctuations in both carotene and vitamin A, the variations with season being, however, in the opposite direction to those recorded in the literature for European and American butters (e.g. Morgan & Pritchard (2) and Baumann & Steenbock (3)). In the case of butters from two representative New Zealand dairying districts (Waikato and Manawatu) maximum levels of both carotene and vitamin A occurred in the late winter and spring (July–October) giving a total vitamin A potency of 42–53 i.u./g. fat. These values decreased through the late spring and summer reaching a minimum level of 33–37 i.u./g. fat in late summer (February) and then rising again through the autumn. The changes in potency were shown to be seasonal rather than lactational. Results, to be published shortly by McDowall & McDowell (4), of an extensive investigation covering other New Zealand dairying districts and extending over several seasons, confirm the trends observed by Barnicoat, while similar fluctuations have been reported by Farrer, Balding, Warren & Miller (5) for a number of districts in Australia where dairying conditions are comparable to those existing in New Zealand.

The clearly defined seasonal fluctuations apparent in European and American butterfat have been shown to depend largely on variations in the carotene content of the feed available to the cows (e.g. (6, 7)) and Barnicoat suggested that the decrease in vitamin A potency of New Zealand butterfat over the summer period might result from a lowering of the carotene content of the grass associated with the hardening up of the pasture following the spring flush.

Little information was available regarding the carotene content of typical New Zealand pastures, but a limited number of assays carried out by the writer on mixed pastures mainly of ryegrass and clover during 1949–50, and a more extensive investigation by Cawley (8), indicated an average value of about 550 μg./g. dry matter over the autumn, winter and spring periods, with a variation of about $\pm$ 100 μg./g. The values decreased during the summer to an average in midsummer of about 200 μg./g. with individual determinations ranging from 152 to 510 μg./g. In all the above cases analyses were carried out on samples of pasture as near as possible representative of what would be consumed

by grazing animals. Except perhaps under abnormal drought conditions, it seems unlikely therefore that the carotene content of New Zealand pastures falls below about 150 μg./g. dry matter even in late summer when the minimum occurs in the vitamin A potency of the butterfat. Assuming an intake of 10–12 kg. dry matter per day for cows on pasture this gives a daily carotene intake of at least 1·5 g. Wilbur, Hilton & Hauge (9) concluded that cows receiving approximately 460 mg. of carotene per day produced milk of maximum vitamin A potency containing about 53 i.u./g. fat (taking 1 Sherman unit as equivalent to 1·4 i.u. (10) and assuming 80 % fat in the butter). Subsequently, Hauge, Westfall, Wilbur & Hilton (7) obtained fat of approximately the same potency with a carotene intake of 300 mg. per day, using dehydrated alfalfa hay or carotene in oil as a source of provitamin. From this work it would also appear that our minimum potency of about 33 i.u./g. might be expected to result from a carotene intake of only 150 mg. per day and that the actual intake of 1·5 g. should give a fat of maximum potency.

It seems therefore that the problem must be that of availability of carotene from the grass and that, as compared with alfalfa hay or carotene in oil, the bulk of the provitamin in our summer pastures is not utilized by dairy cows. A number of workers have shown that there are wide variations in the availability of carotene from different sources (e.g. (11)). While other factors are undoubtedly involved it is well recognized that adequate tocopherol plays an important part in the utilization of carotene (12). It appears possible therefore that the low summer vitamin A potencies might be due to low tocopherol rather than carotene levels in the pasture, and in this communication the effect on the vitamin A potency of the butterfat of feeding tocopherol and later carotene to dairy cows in midsummer is described.

EXPERIMENTAL

Animals and treatment

Three pairs (designated A, B and C) of monozygotic twins were available from the herd of the Dairy Research Institute, Palmerston North, for the project. These animals were grazing a mixed pasture of the ryegrass-white clover-cocksfoot association reasonably typical of the Manawatu, and were at the middle of their first lactation at the time of the experiment. The cows were grade animals and the breed could only be described as predominantly Jersey. The duration of the experiment was kept to a minimum owing to the limited amount of tocopherol and carotene available, and to avoid as far as possible variations in the potency of the butterfat due to climatic changes. The season chosen was a dry one for the Manawatu, and the experiment was commenced in early February 1951 as soon as the vitamin A potency of mixed herd milk, which had been assayed at intervals through the summer (November–February), decreased to 30–35 i.u./g. fat or about the minimum value reported by Barnicoat.

Following a 4-day pre-treatment period when milk samples were collected daily to establish between twin variations, one animal from each pair, A 2, B 2 and C 2, received a supplement of 1 g. of α-tocopherol per day for 14 days (period 1), the corresponding animals A 1, B 1 and C 1 acting as controls. For the following 12 days (period 2) animal A 2 received a supplement of 3 g. α-tocopherol per day and animal C 2, 3 g. α-tocopherol and 300 mg. β-carotene in the form of a 2 % concentrate. In the case of B 2 supplementation was discontinued at the end of period 1 until the vitamin A potency of the fat was the same as that of the control B 1 (6 days). The animal (B 2) then received daily 300 mg. pure

carotene (90% β, 10% α) dissolved in oil for the remainder of period 2 (6 days). During both periods milk samples were collected at 2- or 3-day intervals, all samples being taken at the evening milking.

Methods of assay

The milk samples were separated by gravity creaming. The cream was drawn off and churned and the butter obtained melted and filtered at 60° C. These operations were carried out as rapidly as possible and precautions taken to avoid exposure to light. The filtered fats were stored at 0° C. and assayed as soon as possible.

Vitamin A was estimated using the spectrophotometric method described by McDowell (13). Absorption measurements at 325 mμ on petroleum ether solutions of the non-saponifiable fraction of the fats were corrected for irrelevant absorption using McDowell's formula, corrected density reading at 325 mμ = 9/10 (A − 12/100 B), where A = density reading of non-saponifiable residue in petroleum ether at 325 mμ and B = density reading of the same solution at 450 mμ. This corrected reading was converted to μg. vitamin A using an $E^{1\%}_{1\,cm.}$ value of 1780 for vitamin A alcohol.

Similar petroleum ether solutions of the non-saponifiable residues were used for the carotene estimations, the absorption being measured at 450 mμ after washing with 90% methanol to remove xanthophylls. $E^{1\%}_{1\,cm.}$ for β-carotene in petroleum ether was taken as 2500. A Beckman spectrophotometer was used for all absorption measurements.

In order to compare total vitamin A potencies of the fats, the carotene and vitamin A figures were converted to international units using the factors: carotene i.u./g. = μg./g. × 1·67 (by definition), and vitamin A i.u./g. = μg./g. × 3·2. (This factor of 3·2 for vitamin A was also used by Barnicoat.)

The method used for tocopherols was essentially that described by Quaife (14). Carotene was estimated in pasture samples at intervals during the experiment using a modification (15) of the method of cold extraction with a 'foaming mixture' of petroleum ether and ethanol as described by Moore & Ely (16).

RESULTS

The vitamin A and carotene contents of the butterfat samples collected during the experiment are shown as μg./g. fat in Fig. 1, while total vitamin A potencies expressed as i.u./g. fat are given in Fig. 2.

Pre-feeding period

The samples collected during this period established that although differences in vitamin A and carotene levels attributable to breed differences and individuality occurred between pairs, the between twin differences were small. The average vitamin A potency for the six animals over this period was 34 i.u./g. which agrees with the minimum levels quoted by Barnicoat.

Period 1

From the data presented in Figs. 1 and 2 it is apparent that during this period the supplement of 1 g. of tocopherol per day markedly increased the levels of both vitamin A and carotene in the milk fat. Although again the animals differed in their response to the tocopherol, the effect was definitely apparent in all cases from about the eleventh day of the experiment. A statistical analysis of the vitamin A potencies of the fat samples collected from this date to the end of period 1 (5 collections on alternate days—thirty

samples in all) is summarized in Table 1. In addition to the significance between treatment differences, highly significant differences were found between repeats (successive measurements) and for repeats by treatments. Since from Fig. 2 it is apparent that the untreated animals' potencies were not changing but those of the treated animals were increasing, this significant difference between successive measurements is due to the increasing

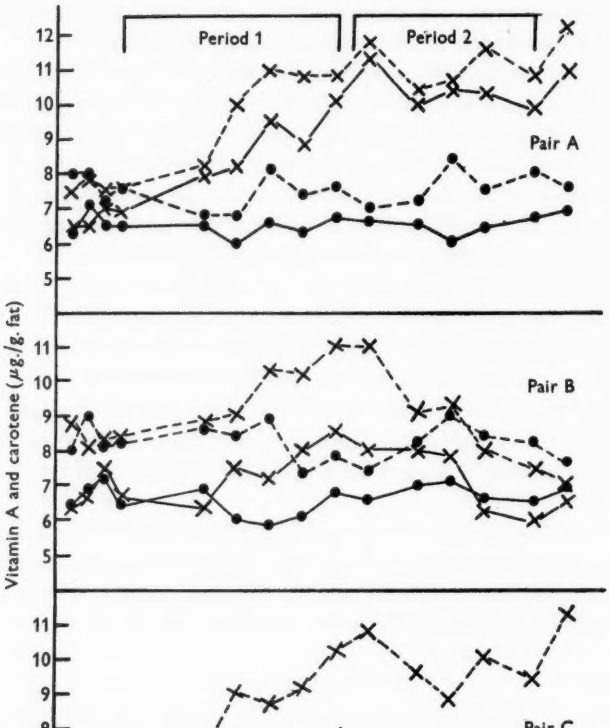

Fig. 1. Carotene and vitamin A in butterfat samples. Carotene: control, ●——●; treated, ×——×. Vitamin A: control, ●---●; treated, ×---×.

effect of the treatment on the vitamin A potency of the fat from the treated animals. The same explanation applies in the case of repeats by treatments, treatment differences being small at the beginning and greater at the end of the period being considered. Towards the end of period 1 the vitamin A potency of the fat from the treated animals approximated the maximum levels reported for typical winter-spring butterfats.

Table 1. *Analysis of vitamin A potencies of fat samples collected from eleventh to nineteenth days inclusive*

Total	Degrees of freedom	Sum of squares	Mean squares	*F*
Between pairs	2	3·5211	1·7606	—
Between treatments	1	13·5609	13·5609	50·02*
Pairs by treatments	2	0·5421	0·2711	—
Between repeats (successive measurements)	4	0·7261	0·1815	5·15**
Repeats by treatments	4	1·1129	0·2782	7·88**
Repeats by pairs	8	0·1644	0·0206	—
Remainder (R × P × T)	8	0·2825	0·0353	—

* Significant 5 % level. ** Significant 1 % level.

Period 2

In view of the results obtained during period 1, supplementation during period 2 was designed to investigate the effect of a higher intake of tocopherol either alone (pair A) or associated with additional carotene (pair C) and of carotene without tocopherol (pair B), and provitamin supplement being supplied in a form previously shown to be normally readily available to dairy cows (7). In the case of pairs A and C, the carotene levels remained high in the fat from the treated animals, no further significant increases resulting from the higher tocopherol or tocopherol and carotene intakes. With pair B, when tocopherol supplementation was stopped at the end of period 1, the potency of the fat from B 2, the previously treated animal, decreased to approximately that of the control within 6 days, and this level was unaffected by feeding 300 mg. of pure carotene in oil (without tocopherol or other anti-oxidant) for a further 6 days, although a maximum potency of about 50 i.u./g. would have been expected from a continued intake of this quantity of carotene in oil (7). During this period of carotene supplementation the vitamin A and carotene levels in the fat from the treated animals actually fell below those of the controls, but the differences are not statistically significant.

Tocopherol supplementation also increased the tocopherol content of the milk fat, and as shown in Fig. 3, a highly significant correlation (+0·57) was established between vitamin A potency and tocopherol content of the fat. In this connexion Krukovsky, Whiting & Loosli (17) found a highly significant correlation between carotene and tocopherol but not between vitamin A and tocopherol.

During the experimental period the carotene content of the pasture grazed by the animals varied from 205 to 490 μg./g. dry matter (average 375 μg./g.), the higher levels being found mainly from the sixteenth day onwards when an increasing amount of fresh pasture was becoming available.

DISCUSSION

The results obtained in this investigation indicate that, as previously calculated from the figures reported by other workers (7, 9), there is adequate carotene in New Zealand summer pastures for the production of milk fat of high vitamin A potency. However, neither this carotene nor the carotene in oil, when given as a supplement to the summer pasture, could be utilized unless the animals also received additional tocopherol, a daily intake of 1 g. of which resulted in the production of fat of maximum potency. It must be concluded therefore that the production of milk fat of high vitamin A potency depends not only on

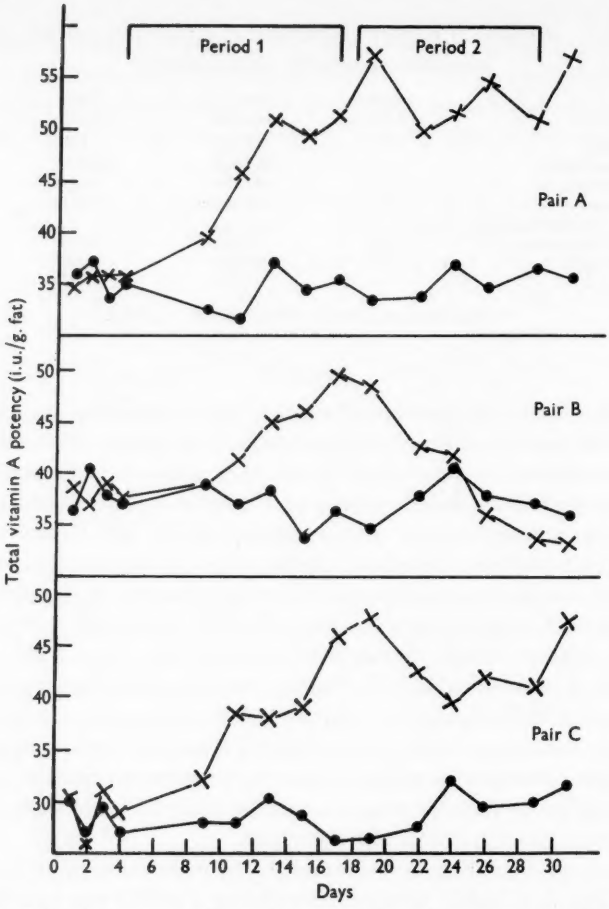

Fig. 2. Total vitamin A potency of butterfat samples. Control, ●——●; treated, ×——×.

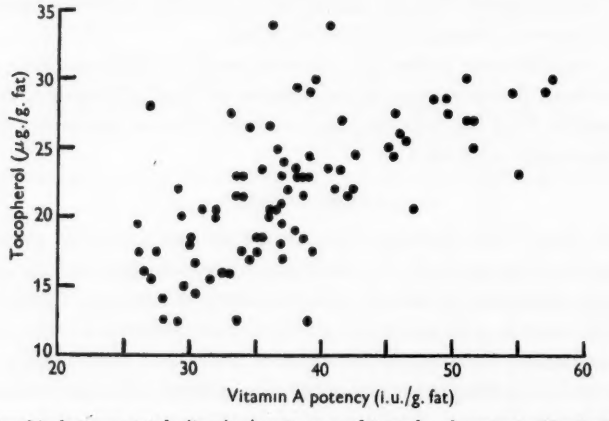

Fig. 3. Relationship between total vitamin A potency and tocopherol content of butterfat samples.

an intake of at least 300 mg. carotene per day but on the presence of adequate tocopherol. Wilbur and his co-workers (7, 9), in their work relating carotene intake to vitamin A potency of the milk fat, did not apparently investigate the tocopherol content of their basal diet. Both in this case and in the case of the investigation reported by Harris, Swanson & Hickman (18), where no increase in the vitamin A potency of the milk fat resulted from the feeding of 1 g. tocopherol per day, it may be assumed that the basal diet already contained sufficient tocopherol for the utilization of the carotene fed.

It is suggested that the low potencies reported for New Zealand butterfats may be due to inadequate tocopherol or other anti-oxidants in the summer pasture. If it is tocopherol which is involved, this must be adequate during the late winter–spring period when potencies are high and the decrease during late spring and summer could be associated with the drying up of the pasture or could be attributable to changes in the botanical composition of the pasture. These changes in composition will depend on the species present and on management, but work at the Grasslands Division, Palmerston North (e.g. (19)) has shown that with clover ryegrass associations the percentage of clover in the sward increases from 10 to 15% in late winter to 50% or higher in summer. In a comparison of hays recently reported by Loosli, Krukovsky, Lofgreen & Musgrave (20), it was found that although ladino clover was higher in carotene content than other hays investigated, the blood plasma and milk fat of cows were lowest in vitamin A value when ladino clover hay was fed.

The effect on the vitamin A potency of milk fat of the high percentage of clover in New Zealand summer pasture is being further investigated in this laboratory and will be reported in a subsequent communication. Preliminary work has established that clover is considerably lower in tocopherol than ryegrass sampled at the same time and that the tocopherol content of mixed pasture, predominantly clover and ryegrass, decreases through the spring reaching a minimum in summer.

SUMMARY

1. Reasons for the seasonal variations in the vitamin A potency of New Zealand butterfat have been investigated. Adequate carotene for the production of butterfat of maximum potency has been shown to be present in New Zealand pasture throughout the year, but during the summer period this carotene is relatively non-available to dairy cows.

2. In experiments with monozygotic twins grazing average summer pasture it has been shown that a supplement of 1 g. of tocopherol per day increased both carotene and vitamin A levels in the milk fat, the total vitamin A potency being raised from a typical midsummer minimum of 34 i.u./g. fat to a value approaching the maximum late winter-spring level of about 50 i.u./g. This maximum level was unaffected by increasing the tocopherol supplement to 3 g. per day or by supplying 300 mg. of carotene in a readily available form in addition to the 3 g. tocopherol.

3. Supplementing the summer pasture with 300 mg. of carotene in oil without tocopherol was without effect on the potency of the fat, supporting the conclusion that the carotene intake was already adequate.

4. A significant correlation was found to exist between vitamin A potency and tocopherol content of New Zealand butterfat.

5. It is suggested that the low vitamin A potencies may be associated with the high percentage of clover in the summer pastures.

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